APPLICATION FOR LETTERS PATENT

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BROAD SPECIFICITY DNA DAMAGE ENDONUCLEASE

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CROSS REFERENCE TO RELATED APPLICATIONS

The present application claims priority from U.S. Patent Application 09/327,984, filed June 8, 1999 which priority claims from U.S. Provisional Application No. 60/088,521, filed June 8, 1998, and from U.S. Provisional Application No. 60/134,752, filed May 18, 1999.

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BACKGROUND OF THE INVENTION

The field of the present invention is the area of DNA repair enzymes. In particular, the invention concerns the identification of stable ultraviolet DNA endonuclease polypeptide fragments, their nucleotide sequences and recombinant host cells and methods for producing them and for using them in DNA repair processes.

The integrity of its genetic material must be maintained in order for a biological species to survive. However, DNA is continuously subject to damage by endogenous and exogenous agents that can lead to mutations, neoplasia or cell death [Smith et al. (1996) *Biochemistry* 35:4146-4154; Brash et al. (1991) *Proc. Natl. Acad. Sci. USA* 88:10124-10128]. One potential source of mutations is nucleotide misincorporation by DNA polymerases during DNA replication

or repair. In addition, primer/template slippage can occur at repetitive DNA sequences during replication, resulting in single-stranded loops of one or more unpaired bases called insertion/deletion loops (IDLs) that can be mutagenic [Sancar, A. (1999) *Nat. Genet.* 21:247-249]. The human genome has an abundance of simple repeat sequences that are relatively unstable [Petruska et al. (1998) *J. Biol. Chem.* 273(9):5204-5210]. Expansion of such repeat sequences have been associated with human genetic diseases including Huntington's disease, fragile X syndrome and myotonic dystrophy [Pearson et al. (1998) *Nucleic Acids Res.* 26(3):816-823].

The *Escherichia coli* Mut HLS pathway has been extensively characterized and is the prototypical DNA mismatch repair (MMR) pathway. This repair pathway recognizes and repairs small IDLs and all single base mismatches except C/C in a strand-specific manner [Modrich, P. (1991) *Annu. Rev. Genet.* 25:229-253]. Mismatch repair pathways have been highly conserved during evolution [Modrich and Lahaue (1996) *Annu. Rev. Biochem.* 65:101-133]. Eukaryotes including *Saccharomyces cerevisiae* and humans have several genes encoding proteins homologous to bacterial MutL and MutS [Sancar, A. (1999) *supra*]. For example, there are six MutS (Msh1-Msh6) and four MutL (MLH1-3, PMS1) homologs in *S. cerevisiae* [Kolodoner, R. (1996) *Genes Dev.* 10:1433-1442]. The Msh2p-Msh6p heterodimer binds base mismatches and small IDLs whereas the Msh2p-Msh3p heterodimer binds only small and large IDLs [Marsischky et al. (1996) *Genes Dev.* 10(4):407-420]. A considerable amount of evidence implicates mismatch repair in stabilizing repetitive DNA sequences [Marsischky et al. (1996) *supra*; Fujii et al. (1999) *J. Mol. Biol.* 289:835-850; Strand et al. (1993) *Nature* 365:274-276].

Cellular exposure to ultraviolet radiation (UV) results in numerous detrimental effects including cell death, mutation and neoplastic transformation. Studies indicate that some of these deleterious effects are due to the formation of two major classes of bipyrimidine DNA photoproducts, cyclobutane pyrimidine dimers (CPDs) and (6-4) photoproducts (6-4 PPs). [Friedberg et al. (1995) In: DNA Repair and Mutagenesis, *Am. Soc. Microbiol.*, Washington, DC, pp. 24-31].

Organisms have evolved several different pathways for removing CPDs and 6-4 PPs from cellular DNA [Friedberg et al. (1995) *supra*; Brash et al. (1991) *supra*]. These pathways include direct reversal and various excision repair pathways which can be highly specific or nonspecific for CPDs and 6-4 PPs. For example, DNA photolyases specific for either CPDs or 6-4 PPs have been found in a variety of species and restore the photoproduct bases back to their original undamaged states [Rubert, C.S. (1975) *Basic Life Sci.* 5A:73-87; Kim et al. (1994) *J. Biol. Chem.* 269:8535-8540; Sancar, G.B. (1990) *Mutat. Res.* 236:147-160]. Excision repair has been traditionally divided into either base excision repair (BER) or nucleotide excision repair (NER) pathways, which are mediated by separate sets of proteins but which both are comprised of DNA incision, lesion removal, gap-filling and ligation reactions [Sancar, A. (1994) *Science* 266:1954-19560; Sancar and Tang (1993) *Photochem. Photobiol.* 57:905-921]. BER N-glycosylase/AP lyases specific for CPDs cleave the N-glycosidic bond of the CPD 5' pyrimidine and then cleave the phosphodiester backbone at the abasic site via a β-lyase mechanism, and have been found in several species including T4 phage-infected *Escherichia coli*, *Micrococcus luteus*, and *Saccharomyces cerevisiae* [Nakabeppu et al. (1982) *J. Biol. Chem.* 257:2556-2562; Grafstrom et al. (1982) *J. Biol. Chem.* 257:13465-13474; Hamilton et al. (1992) *Nature* 356:725-728]. NER is a widely distributed, lesion non-specific repair pathway which orchestrates DNA damage removal via a dual incision reaction upstream and downstream from the damage site, releasing an oligonucleotide containing the damage and subsequent gap filling and ligation reactions [Sancar and Tang (1993) *supra*].

Recently, an alternative excision repair pathway initiated by a direct acting nuclease which recognizes and cleaves DNA containing CPDs or 6-4 PPs immediately 5' to the photoproduct site has been described [Bowman et al. (1994) *Nucleic. Acids Res.* 22:3026-3032; Freyer et al. (1995) *Mol. Cell. Biol.* 15:4572-4577; Doetsch, P.W. (1995) *Trends Biochem. Sci.* 20:384-386; Davey et al.(1997) *Nucleic Acids Res.* 25:1002-1008; Yajima et al. (1995) *EMBO J.* 14:2393-2399; Yonemasu et al. (1997) *Nucleic Acids Res.* 25:1553-1558; Takao et al. (1996) *Nucleic Acids Res.* 24:1267-1271]. The initiating enzyme has been termed UV damage endonuclease (UVDE, now termed Uve1p). Homologs of UVDE have been found in

Schizosaccharomyces pombe, *Neurospora crassa* and *Bacillus subtilis* [Yajima et al. (1995) *supra*; Yonemasu et al. (1997) *supra*; Takao et al. (1996) *supra*]. The Uve1p homologs from these three species have been cloned, sequenced and confer increased UV resistance when introduced into UV-sensitive strains of *E. coli*, *S. cerevisiae*, and human cells [Yajima et al. (1995) *supra*; Takao et al. (1996) *supra*]. In *S. pombe* Uve1p is encoded by the *uve1+* gene. However, because of the apparently unstable nature of partially purified full-length and some truncated UVDE derivatives, UVDE enzymes have been relatively poorly characterized and are of limited use [Takao et al. (1996) *supra*].

Because of the increasing and widespread incidence of skin cancers throughout the world and due to the reported inherent instability of various types of partially purified full-length and truncated UVDE derivatives, there is a long felt need for the isolation and purification of stable UVDE products, especially for use in skin care and medicinal formulations.

SUMMARY OF THE INVENTION

It is an object of the present invention to provide purified stable UVDE (Uve1p), polypeptide fragments which retain high levels of activity, particularly those from the *Schizosaccharomyces pombe* enzyme. In a specific embodiment, the polypeptide fragment is Δ228-UVDE, which contains a 288 amino-acid deletion of the N-terminal region of the *S. pombe uve1+* gene product; a second specific embodiment is the fusion protein GST-Δ288-UVDE. The DNA sequence encoding GST-full-length UVDE from *S. pombe* is given in SEQ ID NO:1. The deduced amino acid sequence of full-length UVDE is given in SEQ ID NO:2. The DNA sequence encoding Δ228-UVDE is given in SEQ ID NO:3. The deduced amino acid sequence of Δ228-UVDE is given in SEQ ID NO:4. The DNA coding sequence and deduced amino acid sequence for GST-Δ228-UVDE are given in SEQ ID NO:5 and SEQ ID NO: 6, respectively. Also encompassed within the present invention are truncated UVDE proteins wherein the truncation is from about position 100 to about position 250 with reference to SEQ ID NO:2, and wherein the truncated proteins are stable in substantially pure form.

Also within the scope of the present invention are nucleic acid molecules encoding such polypeptide fragments and recombinant cells, tissues and animals containing such nucleic acids or polypeptide fragments, antibodies to the polypeptide fragments, assays utilizing the polypeptide fragments, pharmaceutical and/or cosmetic preparations containing the polypeptide fragments and methods relating to all of the foregoing.

A specifically exemplified embodiment of the invention is an isolated, enriched, or purified nucleic acid molecule encoding Δ228-UVDE. Another exemplified embodiment is an isolated, enriched or purified nucleic acid molecule encoding GST-Δ228-UVDE.

In a specifically exemplified embodiment, the isolated nucleic acid comprises, consists essentially of, or consists of a nucleic acid sequence set forth in SEQ ID NO:3 or SEQ ID NO:5.

In another embodiment, the invention encompasses a recombinant cell containing a nucleic acid molecule encoding Δ228-UVDE or GST-Δ228-UVDE. The recombinant nucleic acid may contain a sequence set forth in SEQ ID NO:3 or SEQ ID NO:5, a synonymous coding sequence or a functional derivative of SEQ ID NO:3 or SEQ ID NO:5. In such cells, the Δ228-UVDE coding sequence is generally expressed under the control of heterologous regulatory elements including a heterologous promoter that is not normally coupled transcriptionally to the coding sequence for the UVDE polypeptide in its native state.

In yet another aspect, the invention relates to a nucleic acid vector comprising a nucleotide sequence encoding Δ228-UVDE or GST-Δ228-UVDE and transcription and translation control sequences effective to initiate transcription and subsequent protein synthesis in a host cell. Where a GST full length or truncated derivative is expressed, the GST portion is desirably removed (after affinity purification) by protease cleavage, for example using thrombin.

It is yet another aspect of the invention to provide a method for isolating, enriching or purifying the polypeptide termed Δ228-UVDE.

In yet another aspect, the invention features an antibody (e.g., a monoclonal or polyclonal antibody) having specific binding affinity to a UVDE polypeptide fragment. By "specific binding affinity" is meant that the antibody binds to UVDE polypeptides with greater affinity than it binds to other polypeptides under specified conditions.

Antibodies having specific binding affinity to a UVDE polypeptide fragment may be used in methods for detecting the presence and/or amount of a truncated UVDE polypeptide in a sample by contacting the sample with the antibody under conditions such that an immunocomplex forms and detecting the presence and/or amount of the antibody conjugated to the UVDE polypeptide. Kits for performing such methods may be constructed to include a first container having a conjugate of a binding partner of the antibody and a label, for example, a radioisotope or other means of detection as well known to the art.

Another embodiment of the invention features a hybridoma which produces an antibody having specific binding affinity to a UVDE polypeptide fragment. By "hybridoma" is meant an immortalized cell line which is capable of secreting an antibody, for example a Δ228-UVDE specific antibody. In preferred embodiments, the UVDE specific antibody comprises a sequence of amino acids that is able to specifically bind Δ288-UVDE. Alternatively, a GST-tag specific antibody or labeled ligand could be used to determine the presence of or quantitate a GST-Δ228-UVDE polypeptide, especially in formulations *ex vivo*.

The present invention further provides methods for cleaving DNA molecules at positions with structural distortions, wherein the DNA is cleaved in the vicinity of the distortion by a stable truncated UVDE protein of the present invention. The structural distortion can result from mismatch at the site of the distortion in a double-stranded DNA molecule, from UV damage or from other damage to DNA due to chemical reaction, for example, with an alkylating or depurination agent or due to damage due to UV irradiation, ionizing radiation or other irradiation damage. The stable truncated UVDE proteins can be supplied in substantially pure form for *in vitro* reactions or they can be supplied for *in vivo* reactions, including but not limited to

compositions for topical application (in the form or of an ointment, salve, cream, lotion, liquid or transdermal patch) in pharmaceutical compositions for internal use (to be administered by intraperitoneal, intradermal, subcutaneous, intravenous or intramuscular injection). The stable truncated UVDE derivatives of the present invention repair a wide variety of mismatch and DNA damage. The cleavage of a double stranded DNA molecule having structural distortion due to nucleotide mispairing (mismatch) or due to DNA damage by a stable truncated UVDE derivative of the present invention can be used to advantage in a relatively simple assay for structural distortion wherein cleavage of a test molecule (i.e., the double stranded DNA molecule which is being screened for damage, mismatch or other structural distortion) is to be detected.

The present invention further provides a method for clearing a double stranded DNA molecule in which there is a structural distortion. The structural distortion can be due to aberrations including, but not limited to, base pair mismatch, photoproduct formation, alkylation of a nucleic acid molecule such that normal Watson-Crick base pairing is disturbed, intercalation between nucleotides of a compound which could be, for example, an acriflavine, an ethidium halide, among others, or a platinum adduct, for example of a cisplatin moiety. The distortion can also be an insertion-deletion loop of five or fewer nucleotides. Desirably, the loop has four or fewer nucleotides. The DNA can also contain an abasic site, a uracil residue resulting from deamenation of a cytosine residue, among others. The method of the present invention can be employed using the UVDE (Uve1p) protein from *Schizosaccharomyces pombe*, a truncated derivative of the *S. pombe* UVDE (lacking from about 100 to about 250 N-terminal amino acids), the Δ228-UVDE of *S. pombe*, or the *Neurospora crassa*, *Bacillus subtilis*, *Homo sapiens* or *Deinococcus radiodurans* enzymes as set forth herein (see SEQ ID NOs:36-39). Additional homologs of the *S. pombe* UVDE include the UV damage enzyme of *Bacillus anthracis*; *Halobacterium* sp.; described in Genbank Accession No. AAC 82899; *Methanococcus jannaschii*; disclosed in Genbank Accession No. 057597; and *Thermotoga maritima*, disclosed in Genbank Accession No. AE001740. Specifically exemplified truncated UVDE (Δ228) is given in SEQ ID NO:4. DNA containing the structural distortion is contacted with an enzyme (or

active truncated derivative) as described above under conditions allowing endonucleolytic cleavage of one strand of the distorted DNA molecule.

BRIEF DESCRIPTION OF THE DRAWINGS

Figs. 1A-1C show purification and activity of GST-Δ228-UVDE and Δ228-UVDE. GST-Δ228-UVDE and Δ228-UVDE from overexpressing *S. cerevisiae* DY150 cells were purified by affinity chromatography on glutathione-Sepharose columns. Fig. 1A shows the purification of GST-Δ228-UVDE. Proteins were visualized on a silver-stained 12% SDS-polyacrylamide gel. Lanes M: protein molecular weight markers (sizes indicated on the right). Lane 1: 0.5 μg of soluble protein (column load) from crude extract of *S. cerevisiae* overexpressing cells. Lane 2: 0.5 μg of unbound protein from affinity column flow through. Lane 3: 1.0 μg of unbound protein from column wash fractions. Lanes 4-8: equal volume (20 μL) loads of column fractions from affinity column bound proteins eluted with glutathione corresponding to 5, 15, 65, 55, and 35 ng of total protein, respectively. Fig. 1B illustrates SDS-PAGE analysis (silver-stained 12% gel) of proteins following reapplication of GST-Δ228-UVDE onto glutathione-Sepharose and on-column thrombin cleavage to remove the GST tag. Lane M: protein molecular weight markers (sizes indicated on left). Lane 1: 100 ng of GST-Δ228-UVDE (column load). Lane 2: 250 ng thrombin reference marker. Lane 3: 250 ng of Δ228-UVDE eluted from column following thrombin cleavage. Lane 4: 400 ng (total protein) of GST-Δ228-UVDE and GST remaining bound to affinity column following thrombin cleavage and elution with glutathione. Arrows indicate the positions of GST-Δ228-UVDE (A, 68.7 kDa), Δ228-UVDE (B, 41.2 kDa), thrombin (C, 37 kDa), and GST (D, 27.5 kDa). Fig. 1C shows activities of GST-Δ228-UVDE and Δ228-UVDE preparations on CPD-30mer. CPD-30mer was incubated with the following preparations of UVDE: crude extract of overexpressing cell containing vector alone (lane 1), GST-Δ228-UVDE (lane 2), FL-UVDE (lane 3), affinity-purified GST alone (lane 4), affinity-purified GST-Δ228-UVDE (lane 5) and affinity-purified Δ228-UVDE (lane 6). Oligonucleotide cleavage products (14mer) corresponding to UVDE-mediated DNA strand

scission of CPD-30mer immediately 5' to the CPD site were analyzed on DNA sequencing gels and subjected to autoradiography and phosphorimager analysis.

Fig. 2 shows the effect of salt concentration on UVDE activity. DNA strand scission assays on end-labeled CPD-30mer were carried out with 150 ng of affinity -purified GST-Δ228-UVDE (open circles) or 40 ng of affinity-purified Δ228-UVDE (closed circles) at pH 7.5 and various concentrations of NaCl under otherwise standard reaction conditions for 20 min (See Examples hereinbelow). Extent of DNA strand scission was determined from phosphorimager analysis of gels. Enzyme activity is expressed as a percentage of CPD-30mer cleaved relative to that observed at 100 mM NaCl (defined as 100%).

Fig. 3 illustrates the effect of pH on UVDE activity. DNA strand scission assays on end-labeled CPD-30mer were carried out with 150 ng of affinity -purified GST-Δ228-UVDE (open circles) or 40 ng of affinity-purified Δ228-UVDE (closed circles) under various pH conditions at otherwise standard reaction conditions for 20 minutes (as described herein). Extent of DNA strand scission was determined from phosphorimager analysis of gels and enzyme activity is expressed as a percentage of CPD-30mer cleaved relative to that observed at pH 6.5 (defined as 100%).

Fig. 4 shows the temperature dependence of UVDE activity. DNA strand scission assays on end-labeled CPD-30mer were carried out with 150 ng of affinity -purified GST-Δ228-UVDE (open circles) or 40 ng of affinity-purified Δ228-UVDE (closed circles) at the indicated temperatures under otherwise standard reaction conditions (See the Examples herein below) for 20 minutes. Extent of DNA strand scission was determined from phosphorimager analysis of gels and enzyme activity is expressed as a percentage of CPD-30mer cleaved relative to that observed at 30°C (defined as 100%).

Figs. 5A-5B illustrates kinetic analysis of CPD-30mer cleavage by purified Δ228-UVDE. Δ228-UVDE (5 nM) was reacted with increasing amounts of 5'-end-labeled CPD-30mer and

analyzed for DNA strand scission as described in the Examples. Fig. 5A is a plot of reaction rate (Rate) vs substrate concentration using the mean ± standard deviation from three separate experiments. Curve shown is the best fit to the Michaelis-Menten equation of the averaged data. Fig. 5B is a Lineweaver-Burk plot of the kinetic data.

Fig. 6A-6B show sites of Uve1p cleavage of CPD containing substrates. Various Uve1p preparations were incubated with 5' or 3' end-labeled (*) cs-CPD-30mer. Cleavage products corresponding to Uve1p-mediated strand scission of cs-CPD-30mer were visualized on a DNA sequencing-type gel. Fig. 6A: 5' end labeled cs-CPD-30mer duplex was incubated with buffer only (lane 1), an extract of cells over-expressing GΔ228-Uve1p (5 μg) (lane 2), affinity-purified GΔ228-Uve1p (lane 3) and affinity-purified Δ228-Uve1p (50 ng of each) (lane 4) and affinity-purified GST alone (2 μg) (lane 5). Fig. 6B: 3' end labeled cs-CPD-30mer duplex was incubated with the same Uve1p preparations. Order of lanes is the same as for Fig. 6A. Arrows **a** and **b** indicate the primary and secondary cleavage sites. The photoproduct (T^T corresponds to CPD) containing section of cs-CPD-30mer is shown at the bottom of the figure. For simplicity the complementary strand is not shown.

Fig. 7A-7D demonstrate that GΔ228-Uve1p recognizes 12 different base mismatch combinations. The 3' end labeled oligo series X/Y-31mer (sequence given at bottom, asterisk indicates labeled strand and labeled terminus) was utilized to assess Uve1p cleavage activity on 16 different base pair and base mispair combinations (Table 1B). Base mispairs indicated above numbered lanes with asterisks denoting base on the labeled strand for G-series (Fig. 7A), A-series (Fig. 7B), C-series (Fig. 7C) and T-series (Fig. 7D) treated with purified GΔ228-Uve1p (odd lanes) or mock reactions (even lanes). Reaction products were analyzed on DNA sequencing-type gels. Arrows indicate Uve1p cleavage sites immediately (arrow a), one (arrow b), and two (arrow c) nucleotides 5' to the mismatch site. G and C + T base-specific chemical cleavage DNA sequencing ladders were run in adjacent lanes as nucleotide position markers.

Figs. 8A-8E show Uve1p activity on bipyrimidine UV induced photoproducts. To determine if Uve1p was capable of recognizing a broad spectrum of UV induced photoproducts, crude extracts from cells expressing GΔ228-Uve1p (lane 1) and G-Uve1p (lane 2) (5 μg of each), and affinity-purified Δ228-Uve1p (lane 3) and GΔ228-Uve1p (lane 4) (50 ng of each) were incubated with the following 5' end-labeled (*) duplex oligonucleotide substrates (Fig. 8A) cs-CPD-49mer, (Fig. 8B) 6-4PP-49mer, (Fig. 8C) tsI-CPD-49mer, (Fig. 8D) tsII-CPD-49mer, and (Fig. 8E) Dewar-49mer. The UV photoproduct (T^T) containing section of the sequence is shown at the bottom of the figure. Arrows **a** and **b** indicate the major and minor products formed by Uve1p mediated cleavage. Arrow **uc** indicates the uncleaved substrate. The sequence of the complementary strand is omitted.

Fig. 9 shows Uve1p activity on a platinum-DNA GG diadduct-containing substrate. Affinity-purified GΔ228-Uve1p (lane 4) and Δ228-Uve1p (1-2 μg) (lane 5) were incubated with 5' end-labeled duplex (*) Pt-Gg-32mer. This substrate was also incubated with buffer alone (lane 2), *E. coli* exonuclease III (150 units (Promega)) (lane 3) and affinity-purified GST (2 μg) (lane 6). Maxam and Gilbert sequencing (lane 1) of the oligonucleotide was carried out to identify the site of cleavage. Arrows **c** and **d** indicate the major and minor cleavage sites, respectively. The platinum-DNA GG diadduct containing section of the substrate is shown at the bottom of the figure. The sequence of the complementary strand is omitted.

Fig. 10A shows cleavage of an oligonucleotide substrate containing an AP site by Uve1p. To investigate if Uve1p was capable of cleaving an abasic site in a hydrolytic manner, we prepared a 5' end-labeled (*) abasic substrate, AP-37mer, and incubated this substrate with buffer alone (lane 1), *E. coli* endonuclease III (AP lyase, lane 2), affinity-purified GΔ228-Uve1p and Δ288-Uve1p (2 μg of each) (lanes 3 and 4), extracts of cells over-expressing GΔ288-Uve1p (5 μg) (lane 5), *E. coli* endonuclease IV (hydrolytic AP endonuclease, lane 6) and purified recombinant GST (2 μg) (lane 7). Fig. 10B demonstrates competitive inhibition of AP site recognition and cleavage. To demonstrate that the products generated are as a result of Uve1p-mediated cleavage at the AP site, AP-37mer was incubated with buffer alone (lane 1), *E. coli*

endonuclease IV (lane 2), and affinity-purified GΔ228-Uve1p (2 μg) (lane 3) with 10X and 40X unlabeled cs-CPD-30mer (lanes 4 and 5, respectively) and 10X and 40X unlabeled UD-37mer (lanes 6 and 7, respectively). Arrows **a** and **b** indicate the primary and secondary Uve1p-mediated cleavage products, respectively. Arrow **uc** indicates the uncleaved substrate. A portion of the sequence of the AP substrate is shown at the bottom of the figure. S corresponds to deoxyribose and p corresponds to phosphate. The location of the cleavage sites of endonuclease III (E_{III}) and endonuclease IV (E_{IV}) are also indicated. For simplicity the complementary strand is omitted from the figure.

Figs. 11A-11B characterize the Uve1p-generated DNA strand scission products and activity of full-length Uve1p. Fig. 11A: Analysis of 5' termini of Uve1p-generated DNA cleavage products with *CX/AY-31mer. 3' end labeled oligo with C/A mismatch (sequence on bottom) reacted with GΔ228-Uve1p and then further treated with PNK or CIP as indicated in the (+) and (-) lanes. Lane 1 is buffer treatment only. Arrows a and b indicate sites of Uve1p cleavage. Fig. 11B: Full length Uve1p possesses mismatch endonuclease activity. 5' end labeled duplex *CX/AY-31mer was incubated with crude extracts of cells expressing either full-length, GST-tagged Uve1p (GFL-Uve1p) (lane 1), truncated Uve1p (GΔ228-Uve1p) (lane 2), cells expressing the GST tag alone (lane 3) or with *E. coli* endonuclease V, a known mismatch endonuclease (lane 4). Arrows indicate cleavage sites immediately (arrow a) and one nucleotide 5' to the mismatch site. Arrow V indicates *E. coli* endonuclease V cleavage 3' to the mismatch site and was used as a position reference. Bands below arrows (indicated by asterisks) correspond to shortened products due to a weak 5' to 3' exonuclease activity present in the Uve1p preparations.

Fig. 12 shows that GΔ228-Uve1p mismatch endonuclease and GΔ228-Uve1p UV photoproduct endonuclease compete for the same substrates. GΔ228-Uve1p was incubated with 3'-end-labeled duplex *CX/AY-31mer (Table 1) in the presence of increasing amounts of unlabeled duplex CPD-30mer (squares) or duplex GX/CY-31mer (triangles) or duplex CX/AY-31 mer (circles). The Uve1p-mediated DNA cleavage products were analyzed on DNA

sequencing gels, and the extent of strand scission was quantified by PhosphorImager analysis. Uve1p activity is expressed as the percentage of the cleavage observed relative to that observed in the absence of any competitor (defined as 100% activity). The error bars indicate the mean ± standard deviation from three separate experiments.

Figs. 13A-13B show that Uve1p incises only one strand of a duplex containing a base mismatch. Fig. 13A shows 3'-end-labeled *CX/AY-41mer incubated with restriction enzyme *Dde*I (lane 1), GΔ228-Uve1p (lane 2), or buffer (lane 3). The reaction products were analyzed on a nondenaturing gel as described below for the presence of DNA double-strand break products (arrow dsb). Arrows b and c indicate the primary cleavage site for Uve1p on this substrate. Fig. 13B shows 3'-end-labeled *CX/AY-41mer or CX/*AY-41mer incubated with GΔ228-Uve1p (+ lanes) or buffer (- lanes) and analyzed on denaturing DNA sequencing-type gels. Arrows b and c indicate positions of major Uve1p cleavage events relative to the mismatched base (asterisk) position. G + A and C + T base-specific sequencing ladders are included in outside lanes as nucleotide position markers.

Figs. 14A-14C show that Uve1p recognizes small IDLs but not large IDLs or hairpin structures. Cleavage reactions were carried out with 3'-end-labeled DNA duplexes under standard reaction conditions and products were analyzed on DNA sequencing gels as described in the text. Arrows (a, b and c) indicate GΔ228-Uve1-mediated DNA cleavage products. G and C+T base-specific chemical cleavage DNA sequencing ladders were run in adjacent lanes as nucleotide position markers. The 3' end labeled (*) core substrate sequence is shown at the bottom of the figure with Uve1p cleavage sites a, b and c indicated (X corresponds to IDL sequences shown in Fig. 14A. Fig. 14 A: 3'-end labeled duplexes ID0, ID2 and ID4; Fig. 14B: ID0 and HP8; (C) ID4, ID6 and ID8 were treated with GΔ228-Uve1p (even numbered (+) lanes) or buffer (odd numbered (-) lanes).

Figs. 15A-15B:15 show that Uve1p recognizes short IDL structures within a different sequence context. Cleavage reactions were carried out with (Fig. 15A) 3'-end-labeled or (Fig.

15B) 5'-end-labeled DNA duplexes containing IDLs of 0 (LD0), 2 (LD2) and 4 (LD4) nt in length and GΔ228-Uve1p as described in the text. DNA cleavage products were analyzed on sequencing type gels. Arrows d, e and f indicate DNA strand scission products produced by cleavage events occurring 1, 15 and 16 nt 5' from the IDL site, respectively. G and C+T base-specific chemical cleavage DNA sequencing ladders were run in adjacent lanes as nucleotide position markers. The core sequence of the substrate is shown at the bottom of the figure where X corresponds to the IDL sequence in Table 17A-17B. In Table 17A, the vertical bracket above arrows e and f indicates non-specific cleavage of the substrates and is not observed with 5' end labeled substrates.

Fig. 16 shows antibody inhibition of Uve1p-mediated activity in an IDL containing substrate. GΔ228-Uve1p was pre-incubated with increasing amounts (40 μg - 160 μg) of immune serum (lanes 2-5) or pre-immune serum (lanes 6-9) prior to incubation with 3' end labeled substrate ID2 (sequence indicated at the bottom). The reaction products were analyzed on a 20% denaturing polyacrylamide gel as described (Example). Arrows b and c correspond to GΔ228-Uve1p-mediated DNA cleavage events 15 and 16 nt 5' to the IDL site, respectively. In this gel the two bands representing distinct cleavage events at positions b and c are poorly resolved from each other.

Figs. 17A-17C show the strand specificity of Uve1p is influenced by the proximity of the mispaired base to the 3'-terminus. DNA substrates used in this experiment are shown in the Table 1. Number and letter designations adjacent to substrates correspond to the oligonucleotide names given in Table 17A-17B. Duplex substrates were 3'-end-labeled (*) on (Fig. 17A) C-containing or (Fig. 17B) A-containing strands and incubated with GΔ228-Uve1p as described below. The extent of DNA strand scission (expressed as percent of total substrate cleaved) was quantified by phosphorimager analysis. (Fig. 17C) Substrates AB, AC, AE and AD were 3'-end labeled and incubated with GΔ228-Uve1p under standard conditions as described below. The percent cleavage was quantified by phosphorimager analysis. The error bars indicate the mean ± standard deviation obtained from three separate experiments.

DETAILED DESCRIPTION OF THE INVENTION

Abbreviations used in the present specification include the following: aa, amino acid(s); AER, alternative excision repair; bp, base pair(s); BER, base excision repair; cDNA, DNA complementary to RNA; CPD, cyclobutane pyrimidine dimer; FL, full-length; GΔ228Uve1p, GST-tagged truncated Uve1p; GST, glutathione-S-transferase; IDL, insertion deletion loop; NER, nucleotide excision repair; nt, nucleotide; PAGE, polyacrylamide gel electrophoresis; PMSF, phenylmethanesulfonyl fluoride, 6-4 PP, (6-4) photoproduct; UVDE or Uve1p, used interchangeably, ultraviolet damage endonuclease; Δ228-UVDE, UVDE truncation product lacking 228 N-terminal amino acids.

By "isolated" in reference to a nucleic acid molecule it is meant a polymer of 14, 17, 21 or more nucleotides covalently linked to each other, including DNA or RNA that is isolated from a natural source or that is chemically synthesized. The isolated nucleic acid molecule of the present invention does not occur in nature. Use of the term "isolated" indicates that a naturally occurring or other nucleic acid molecule has been removed from its normal cellular environment. By the term "purified" in reference to a nucleic acid molecule, absolute purity is not required. Rather, purified indicates that the nucleic acid is more pure than in the natural environment.

A "nucleic acid vector" refers to a single or double stranded circular nucleic acid molecule that can be transfected or transformed into cells and replicate independently or within the host cell genome. A circular double stranded nucleic acid molecule can be linearized by treatment with the appropriate restriction enzymes based on the nucleotide sequences contained in the cloning vector. A nucleic acid molecule of the invention can be inserted into a vector by cutting the vector with restriction enzymes and ligating the two pieces together. The nucleic acid molecule can be RNA or DNA.

Many techniques are available to those skilled in the art to facilitate transformation or transfection of the recombinant construct into a prokaryotic or eukaryotic organism. The terms

"transformation" and "transfection" refer to methods of inserting an expression construct into a cellular organism. These methods involve a variety of techniques, such as treating the cells with high concentrations of salt, an electric field, or detergent, to render the host cell competent for uptake of the nucleic acid molecules of interest or liposome-mediated transfection can be employed.

The term "promoter element" describes a nucleotide sequence that is incorporated into a vector which enables transcription, in appropriate cells, of portions of the vector DNA into mRNA. The promoter element precedes the 5' end of the Δ228-UVDE or GST-Δ228-UVDE nucleic acid molecule such that the Δ228-UVDE OR GST-Δ228-UVDE sequence is transcribed into mRNA. Transcription enhancing sequences may also be incorporated in the region upstream of the promoter. mRNA molecules are translated to produce the desired protein(s) within the recombinant cells.

Those skilled in the art would recognize that a nucleic acid vector can contain many other nucleic acid elements besides the promoter element and the Δ228-UVDE or GST-Δ228-UVDE nucleic acid molecule. These other nucleic acid elements include, but are not limited to, origins of replication, ribosomal binding sites, transcription and translation stop signals, nucleic acid sequences encoding drug resistance enzymes or amino acid metabolic enzymes, and nucleic acid sequences encoding secretion signals, periplasm or other localization signals, or signals useful for polypeptide purification.

As used herein, "Δ228-UVDE polypeptide" has an amino acid sequence as given in or substantially similar to the sequence shown in SEQ ID NO:4. A sequence that is substantially similar will preferably have at least 85% identity and most preferably 99-100% identity to the sequence shown in SEQ ID NO:4. Those skilled in the art understand that several readily available computer programs can be used to determine sequence identity with gaps introduced to optimize alignment of sequences being treated as mis-matched amino acids and where the sequence in SEQ ID NO:4 is used as the reference sequence.

As used herein, "GST-Δ228-UVDE polypeptide (GΔ228Uve1p) has an amino acid sequence as given in or substantially similar to the sequence shown in SEQ ID NO:6. A sequence that is substantially similar will preferably have at least 85% identity and most preferably 99-100% identity to the sequence shown in SEQ ID NO:6. Those skilled in the art understand that several readily available computer programs can be used to determine sequence identity with gaps introduced to optimize alignment of sequences being treated as mis-matched amino acids and where the sequence in SEQ ID NO:6 is used as the reference sequence.

By "isolated" in reference to a polypeptide is meant a polymer of 6, 12, 18 or more amino acids conjugated to each other, including polypeptides that are isolated from a natural source or that are chemically synthesized. The isolated polypeptides of the present invention are unique in the sense that they are not found in a pure or separated state in nature. Use of the term "isolated" indicates that a naturally occurring sequence has been removed from its normal cellular environment. Thus, the sequence may be in a cell-free solution or placed in a different cellular environment. The term does not imply that the sequence is the only amino acid chain present, but that it is essentially free (at least about 90-95% pure) of material naturally associated with it.

The term "purified" in reference to a polypeptide does not require absolute purity (such as a homogeneous preparation); instead, it represents an indication that the polypeptide is relatively purer than in the natural environment. Purification of at least two orders of magnitude, preferably three orders of magnitude, and more preferably four or five orders of magnitude is expressly contemplated, with respect to proteins and other cellular components present in a truncated UVDE-containing composition. The substance is preferably free of contamination at a functionally significant level, for example 90%, 95%, or 99% pure. Based on increases in calculated specific activity, GST-Δ228-UVDE and Δ228-UVDE have been purified 230-fold and 310-fold, respectively. However, based on silver-stained SDS polyacrylamide gel results, it appears that both proteins have been purified nearly to homogeneity (see Fig.1).

As used herein, a "UVDE polypeptide fragment" or "truncated UVDE" has an amino acid sequence that is less than the full-length amino acid sequence shown in SEQ ID NO:2. Also as used herein, UVDE and Uve1p are used synonymously.

In the present context, a "UVDE mutant polypeptide" is a UVDE polypeptide or truncated UVDE which differs from the native or truncated native sequence in that one or more amino acids have been changed, added or deleted. Changes in amino acids may be conservative or non-conservative. By conservative it is meant the substitution of an amino acid for one with similar properties such as charge, hydrophobicity and structure. A UVDE mutant polypeptide of the present invention retains its useful function, i.e., for example, ability to remove cyclobutane pyrimidine dimers and/or (6-4) photoproducts from DNA, and its enzymatic activity is stable in its substantially purified form. The full-length UVDE protein and the truncated derivatives of the present invention recognize a wide variety of DNA damage and distortions to double stranded DNA, as described hereinbelow. The UVDE and truncated UVDE proteins are useful in cleaving double-stranded DNA molecules in which damage including but not limited to abasic sites, photoproducts, cis-platin adducts and a variety of other aberrations also including mismatched base pairing and sites adjacent to and at locations of intercalations (for example with acridine dyes or ethidium bromide, among others, and these proteins, particularly the stable truncated derivatives of the present invention are useful in vivo and/or in vitro for repairing DNA distortions as described herein.

The isolation of genes encoding UVDEs from different organisms has been described previously [Yajima et al. (1995) *supra*; Takao et al. (1996) *supra*]. These genes have been cloned by introducing a foreign cDNA library into a repair-deficient *E. coli* strain and selecting for complemented cells by UV irradiation of the transformants. [Yajima et al. (1995) *supra*; Takao et al. (1996) *supra*]. Researchers have not characterized full-length UVDEs because they become unstable and lose their activity when purified [Takao et al. (1996) *supra*]. This instability makes their use as therapeutic agents problematical.

Because UVDEs can be used for a variety of applications including the treatment and prevention of diseases caused by DNA damage, the inventors sought to discover stable UVDEs. The present inventors have noted that the activity of the full-length UVDE appears relatively stable to storage and freeze-thawing when it is present in crude extracts of either its native *Schizosaccharomyces pombe* or recombinant *Escherichia coli* [see also Takao et al. (1996) *supra*]. The present inventors and others have not had success in obtaining enzymatically active purified UVDE in good yield. The present invention describes the isolation and purification of a polypeptide fragment from *S. pombe* which exhibits superior stability and enzymatic activity than purified full-length UVDE.

The full-length *uvde* gene from *S. pombe* was amplified from a cDNA library by the polymerase chain reaction (PCR) using methods known to those skilled in the art and as described herein. Δ228-UVDE, which contains a deletion of the of the first 228 N-terminal amino acids of full-length UVDE, was prepared using PCR as described herein.

The amplified UVDE gene coding fragments were cloned into the yeast expression vector pYEX4T-1. In pYEX 4T-1, the UVDE-derived polypeptides are expressed in frame with a glutathione-S-transferase (GST) leader sequence to generate a fusion protein of GST linked to the N-terminus of UVDE. The DNA sequence of the GST leader is shown in SEQ ID NO:7. The deduced amino acid sequence of the GST leader is shown in SEQ ID NO:8. Appropriate plasmids containing the DNA fragments in the proper orientation were transformed into *S. cerevisiae*, DY150 cells using the alkali cation method [Ito et al. (1993) *J. Bacteriol.* 153:163-163]. Positive clones were selected and used for protein purification.

Both full-length UVDE and Δ228-UVDE were isolated and purified using glutathione-Sepharose affinity chromatography. Extracts from cells expressing GST-Δ228-UVDE were passed through glutathione-Sepharose columns. GST-Δ228-UVDE which bound to the column was eluted using glutathione. Additionally, Δ228-UVDE was generated by removal of the GST-leader from GST-Δ228-UVDE by treating GST-Δ228-UVDE, which had bound to the

glutathione-Sepharose column, with thrombin. Pooled fractions from the affinity purification yielded approximately 1.5 mg of near-homogeneous or homogeneous GST-Δ228-UVDE protein per 500 mL of *S. cerevisiae* cells.

GST-Δ228-UVDE and Δ228-UVDE have electrophoretic mobilities corresponding to protein sizes, as determined by SDS-PAGE, of 68.7 kDa and 41.2 kDa, respectively (Fig. 1A, lanes 4-8; Fig. 1B, lane 3). Both crude and purified preparations of Δ228-UVDE and GST-Δ228-UVDE retained enzymatic activity on an oligodeoxynucleotide substrate (CPD-30mer) containing a single cis-syn cyclobutane pyrimidine dimer embedded near the center of the sequence (Fig. 1C). In contrast, purified full-length UVDE resulted in a preparation that was not stable in that enzymatic activity was rapidly lost (Fig. 1C, lane 3). Furthermore, purified GST-Δ228-UVDE and Δ228-UVDE are stable when stored at -80°C in 10% glycerol for a period of at least six months with no substantial loss of activity. Preparations of GST-Δ228-UVDE and Δ228-UVDE are resistant to several rounds of freeze-thawing. Surprisingly, both purified GST-Δ228-UVDE and Δ228-UVDE are more stable and have higher enzymatic activity than purified full-length UVDE.

Both truncated forms of UVDE (GST-Δ228-UVDE and Δ228-UVDE) retained high levels of activity over a broad NaCl concentration range (50-300mM) with an optimum around 100mM (Fig. 2). Optimal cleavage of an oligodeoxynucleotide substrate (CPD-30mer) occurred in the presence of 10mM $MgCl_2$ and 1 mM $MnCl_2$. Both GST-Δ228-UVDE and Δ228-UVDE showed optimal cleavage of CPD-30mer at pH 6.0-6.5 with activity sharply declining on either side of this range indicating that the GST tag does not affect the folding and activity of the protein (Fig. 3). The calculated pI values for GST-Δ228-UVDE and Δ228-UVDE are 6.8 and 7.5, respectively.

Under optimal pH, salt and divalent cation conditions, GST-Δ228-UVDE and Δ228-UVDE were found to exhibit a temperature optimum at 30°C (Fig. 4). At 37°C GST-Δ228-

UVDE and Δ228-UVDE activities decreased to approximately 85% and 60%, respectively and at 65°C, both truncated versions of UVDE showed a significant decrease in activity.

The kinetic parameters for homogeneous GST-Δ228-UVDE and Δ228-UVDE were determined using the CPD-30mer substrate. Fig. 5 shows that Michaelis-Menten kinetics apply to the CPD-30mer cleavage reactions with Δ228-UVDE. Fig. 5B is a Lineweaver-Burk plot of the kinetic data in Fig. 5A. The apparent K_m for CPD-30mer was calculated to be 49.1 nM ± 7.9 nM for GST-Δ228-UVDE and 74.9 nM ± 3.6 nM for Δ228-UVDE. The V_{max} values (nM min^{-1}) were found to be 2.4 ± 0.13 and 3.9± 0.12 for GST-Δ228-UVDE and Δ228-UVDE, respectively. The turnover numbers (K_{cat}) were 0.21 ± 0.01 min^{-1} for GST-Δ228-UVDE and 0.9 ± 0.03 min^{-1} for Δ228-UVDE.

Uve1p has been shown to be capable of recognizing both *cis-syn* CPDs (cs-CPD) and 6-4PPs [Bowman et al. (1994) *Nucl. Acids Res.* 22:3036-3032; Yajima et al. (1995) *EMBO J.* 14:2393-2399]. It is unique in this respect as no other single polypeptide endonuclease is known to recognize both of these UV photoproducts. CPDs and 6-4PPs are the most frequently occurring forms of UV-induced damage, but there are significant differences in the structural distortions induced in DNA by these two lesions. Incorporation of a cs-CPD into duplex DNA causes no significant bending or unwinding of the DNA helix [Rao et al. (1984) *Nucl. Acids Res.* 11:4789-4807; Wang et al. (1991) *Proc. Natl. Acad. Sci. USA* 88:9072-9076; Miaskiewicz et al. (1996) *J. Am. Chem. Soc.* 118:9156-9163; Jing et al. (1998) *supra*; McAteer et al. (1998) *J. Mol. Biol.* 282:1013-1032; Kim et al. (1995) *supra*] and destabilizes the duplex by ~1.5 kcal/mol [Jing et al. (1998) *Nucl. Acids Res.* 26:3845-3853]. It has been demonstrated that this relatively small structural distortion allows CPD bases to retain most of their ability to form Watson-Crick hydrogen bonds [Jing et al. (1998) *supra*; Kim et al. (1995) *Photochem. Photobiol.* 62:44-50]. On the other hand, NMR studies have suggested that 6-4PPs bend the DNA to a greater extent than cs-CPDs, and there is a destablization of ~6 kcal/mol in the DNA duplex with a resulting loss of hydrogen bond formation at the 3'-side of the 6-4PP DNA adduct [Kim et al. (1995) *Eur.*

J. Biochem. 228:849-854]. The ability of Uve1p to recognize such different structural distortions suggests that it might also recognize other types of DNA damage.

CPDs can occur in DNA in four different isoforms (*cis-syn* I [cs I], *cis-syn* II [cs II], *trans-syn* I [ts I] and *trans-syn* II [ts II]) [Khattak and Wang (1972) *Tetrahedron* 28:945-957]. Pyrimidine dimers exist predominately in the cs I form in duplex DNA whereas *trans-syn* (ts) dimers are found primarily in single stranded regions of DNA. 6-4PPs are alkali labile lesions at positions of cytosine (and much less frequently thymine) located 3' to pyrimidine nucleosides [Lippke et al. (1981) *Proc. Natl. Acad. Sci. USA* 78:3388-3392]. 6-4PPs are not stable in sunlight and are converted to their Dewar valence isomers upon exposure to 313 nm light. We have investigated the specificity of Δ228-Uve1p for a series of UV photoproducts: cs-CPD, ts I-CPD, ts II-CPD, 6-4PP and the Dewar isomer. We also investigated the possibility that Uve1p may recognize other types of non-UV photoproduct DNA damage. We describe the activity of Uve1p on DNA oligonucleotide substrates containing a variety of lesions including a platinum-DNA GG diadduct (Pt-GG), uracil (U), dihydrouracil (DHU), 8-oxoguanine (8-oxoG), abasic sites (AP site), inosine (I), and xanthine (Xn). This collection of substrates contains base lesions that induce a broad range of different DNA structural distortions.

Uve1p isolated from *S. pombe* was first described as catalyzing a single ATP-independent incision event immediately 5' to the UV photoproduct, and generating termini containing 3' hydroxyl and 5' phosphoryl groups [Bowman et al. (1994) *Nucl. Acids Res.* 22:3026-3032]. The purified GΔ288-Uve1p, Δ288-Uve1p and crude cell lysates of recombinant G-Uve1p and GΔ288-Uve1p make an incision directly 5' to CPDs similar to that observed with the native protein. In this study, we have used both 5' and 3' end-labeled duplex CPD-30mer (cs-CPD-30mer) to demonstrate the ability of Uve1p to cleave a CPD-containing substrate at two sites (Fig. 6A-6B). The primary product (arrow a) accounted for approximately 90% of the total product formed and resulted from cleavage immediately 5' to the damage. The second incision site was located one nucleotide upstream and yielded a cleavage product (arrow b), which represented the remaining 10% of the product formed. This minor product is one nucleotide

shorter or longer than the primary product depending on whether 5' or 3' end-labeled substrate is being examined. The same cleavage pattern was observed for each different Uve1p preparation used: i.e., crude extracts of cells expressing GΔ228-Uve1p, affinity-purified GΔ228-Uve1p and Δ228-Uve1p (Fig. 2A and 2B, lanes 2, 3 and 4 respectively), as well as extracts of cells expressing GST-Uve1p. No cleavage products were observed when the cs-CPD-30mer substrates were incubated with buffer only, or purified recombinant GST prepared and affinity-purified in an identical manner to the purified Uve1p proteins (Fig. 6A, 6B, lanes 1 and 5 respectively). This control eliminates the possibility that these DNA strand scission products are formed as a result of the presence of trace amounts of non-specific endonuclease contamination. Uve1p recognizes a duplex cs-CPD-containing oligonucleotide substrate and cleaves this substrate at two sites. The primary site, responsible for 90% of the product, is immediately 5' to the damage and the secondary site (accounting for the remaining 10% of product), is one nucleotide 5' to the site of damage.

Uve1p cleaves both CPDs and 6-4PPs when they are incorporated into oligonucleotide substrates [Bowman et al. (1994) *supra*; Yajima et al. (1995) *EMBO J.* 14:2393-2399]. These lesions induce substantially different distortions in duplex DNA. The ability of native Uve1p to recognize both of these damages prompted us to investigate whether this endonuclease recognized other forms of UV-induced photodamage, as well. In order to determine the substrate range of recombinant Δ228-Uve1p for UV-induced bipyrimidine photoproducts, various Uve1p preparations were incubated with synthetic 49-mer oligonucleotides containing different forms of UV damage (Table 1A). The substrates used in these experiments were 5' end labeled duplex cs-CPD-49mer, tsI-CPD-49mer, tsII-CPD-49mer, 6-4PP-49mer and Dewar-49mer (Fig. 8A). Generally, purified GΔ228-Uve1p and Δ228-Uve1p cleaved all of the bipyrimidine photoproduct substrates in a similar manner with respect to both the site and extent of cleavage. The cleavage pattern observed when crude cell lysates of G-Uve1p and GΔ228-Uve1p were incubated with the substrates was less consistent. Very low levels of product were observed when these extracts were incubated with the Dewar isomer. No cleavage products were detected when the damaged substrates were incubated with buffer alone or purified recombinant GST, demonstrating that no

other DNA repair proteins were responsible for the cleavage of the substrate. In addition, incubation of Uve1p with end-labeled undamaged substrate (UD-30mer) did not result in the formation of any cleavage products. We concluded that Uve1p recognizes and cleaves these five UV-induced bipyrimidine photoproducts in a similar manner and that they are substrates for this enzyme. This is the first time that a single protein endonuclease capable of recognizing such a surprisingly broad range of UV-induced photoproducts has been described.

To explore activity on DNA with non-UV-photoproduct diadducts we investigated whether Uve1p recognized an oligonucleotide containing a platinum-DNA lesion. *cis*-Diamminedichloroplatinum(II) (cisplatin) is a widely used antitumor drug that induces several types of mono- and diadducts in DNA. One of the major, biologically relevant adducts formed results from the coordination of N-7 of two adjacent guanines to platinum to form the intrastrand crosslink *cis*-[$Pt(NH_3)_2${d(GpG)-N7(1),-N7(2)}] (*cis*-PT-GG) (Fig. 9). A 5' end-labeled duplex 32-mer oligonucleotide with a single platinum intrastrand crosslink between positions 16 and 17 (Pt-GG-32mer) (Table 1A) was incubated with either GΔ228-Uve1p or Δ228-Uve1p, and the reaction products were visualized on a DNA sequencing-type gel (Fig. 9). The 3' to 5' exonuclease activity of *E. coli* exonuclease III was used to identify the specific site of cleavage of Uve1p, as a platinum-DNA diadduct will terminate or stall the digestion of the duplex DNA at this site [Royer-Pokora et al. (1981) *Nucl. Acids Res.* 9:4595-4609; Tullius and Lippard (1981) *J. Am. Chem. Soc.* 103:4620-4622]. Incubation of 5' end-labeled Pt-GG-32mer with exonuclease III (Fig. 9, lane 3) generates 5' end-labeled oligonucleotide fragments with 3' hydroxyl termini. Maxam and Gilbert sequencing (Fig. 9, lane 1) of the same substrate generates 5' end labeled fragments with 3' phosphoryl termini which consequently migrate faster than the exonuclease III product on DNA sequencing-type gels. (Due to overreaction with hydrazine all of the nucleotides are highlighted in the sequencing lane.) GΔ228-Uve1p cleaved Pt-GG-32mer 5' to the GpG adduct position at two adjacent sites (Fig. 9, lane 4, arrows c and d). The products c) and d) migrate with the exonuclease III products, confirming that they have 3' hydroxyl termini. Comparison with the Maxam and Gilbert sequencing ladder (Fig. 9, lane 1) indicates that the GΔ228-Uve1p-mediated cleavage products are generated by cleavage at sites located two and

three nucleotides 5' to the platinum DNA-GG diadduct. The GΔ228-Uve1p-mediated cleavage products were quantified by phosphorimager analysis, and it was determined that cleavage at the primary site c (arrow c) accounted for approximately 90% of the total product formed while cleavage at the secondary site (arrow d) accounted for the remaining 10%. In contrast, Δ228-Uve1p appeared to cleave Pt-GG-32mer only at the primary site c (i.e., two nucleotides 5' to the damage) (Fig. 9, lane 5). When the quantity of protein used and the total amount of product formed is taken into account, the cleavage of Pt-GG-32mer by Uve1p appears at least 100-fold less efficient than the cleavage of the UV-induced photoproducts. Despite this significant decrease in efficiency, Pt-GG-32mer is a substrate for Uve1p, albeit a poor one, and more importantly, Uve1p is capable of recognizing and cleaving a non-UV photoproduct dimer lesion.

Uve1p is active on substrates containing non-bulky DNA damages. The ability of Uve1p to recognize and cleave non-UV photoproduct DNA diadducts prompted us to investigate whether other types of base damage could also be recognized by this versatile endonuclease. These damages included abasic sites (AP sites), uracil (U), dihydrouracil (DHU), inosine (I), xanthine (Xn) and 8-oxoguanine (8-oxoG) (Scheme 1C). For these studies, we utilized 37-mer oligonucleotide substrates with the damages placed near the center of the molecule and within the same DNA sequence context (Table 1B). These oligonucleotides, Ap-37mer, U-37mer, DHU-37mer and 8-oxoG-37mer were incubated with various Uve1p preparations, and the reaction products were analyzed on DNA sequencing-type gels. In addition, 31mer oligonucleotides containing inosine (I-31mer) and xanthine (Xn-31mer) were also tested as potential Uve1p substrates (Table 1A).

Abasic sites (AP sites) arise in DNA from the spontaneous hydrolysis of N-glycosyl bonds and as intermediates in DNA glycosylase-mediated repair of damaged bases [Sakumi and Sekiguchi (1990) *Mutat. Res.* 236:161-172]. AP endonucleases cleave hydrolytically 5' to the site to yield a 3'hydroxyl termini, AP lyases cleave by a β-elimination mechanism leaving a 3'-αβ-unsaturated aldehyde [Spiering and Deutsch (1981) *J. Biol. Chem.* 261:3222-3228]. To determine if Uve1p cleaves AP sites, we incubated affinity-purified GΔ228-Uve1p and Δ228-

Uve1p and crude extracts of cells expressing GΔ228-Uve1p with a 5' end-labeled oligonucleotide substrate containing an AP site placed opposite a G residue (AP/G-37mer). The products were analyzed on a DNA sequencing-type gel as before (Fig. 10A, lanes 3, 4 and 5 respectively). *E. coli* endonuclease III (which has an associated AP lyase activity) and *E. coli* endonuclease IV (a hydrolytic AP endonuclease) were used to determine if the cleavage products formed during incubation with Uve1p preparations were due to a β-elimination mechanism or hydrolytic cleavage (Fig. 10A, lanes 2 and 6 respectively). Uve1p recognized the AP site in this oligonucleotide substrate and cleaved it in a similar manner to *E. coli* endonuclease IV. Incubating the Uve1p proteins with an oligonucleotide substrate where the AP site was placed opposite an adenine residue (AP/A-37mer) resulted in no significant change in the amount of cleavage product formed. To further test Uve1p recognition of AP sites, we used unlabeled cs-CPD-30mer as a specific competitor for Uve1p. Addition of 40X unlabeled CPD-30mer to reactions of a 5' end-labeled AP/G-37mer with the purified GΔ228-Uve1p resulted in an ~60% decrease in the amount of product formed. The addition of 40X unlabeled undamaged 30mer (UD-30mer) had no effect on the amount of product observed. Uve1p is capable of recognizing AP sites, and changing the complementary base has little or no effect on the extent of cleavage.

Uracil lesions can occur in DNA by the spontaneous deamination of a cytosine residue. Dihydrouracil is a pyrimidine photoproduct that is formed by the deamination of cytosine with subsequent ring saturation upon exposure to ionizing radiation under anoxic conditions [Dizdaroglu et al. (1993) *Biochemistry* 45:12105-12111]. To determine if Uve1p recognized uracil and dihydrouracil lesions, we incubated various preparations of Uve1p with 3' end-labeled 37mer oligonucleotides containing uracil and DHU residues placed opposite a G (U/G-37mer, DHU/G-37mer). The results of this set of experiments are summarized in Table 2. Purified GΔ228-Uve1p cleaved U/G-37mer and DHU/G-37mer in a typical Uve1p mediated fashion: immediately 5' to the position of the lesion to form a major product, and again one nucleotide 5' to the damaged site to form a minor product, 90% and 10% of the total Uve1p-mediated cleavage products, respectively. By contrast, U-A pairs in double stranded DNA molecules are not substrates for Uve1p or its truncated derivative of the present invention.

Persistence of uracil and DHU lesions through replication may lead to the incorporation of adenine residues opposite the damaged base. To examine if Uve1p were equally efficient at recognizing uracil and DHU when they were base paired with an adenine residue, we constructed the substrates U/A-37mer and DHU/A-37mer. The results obtained from the analysis of Uve1p cleavage of these substrates are summarized in Table 2. No Uve1p mediated cleavage products were observed when crude extracts from cells expressing GΔ228-Uve1p and purified GΔ228-Uve1p were incubated with the U/A-37mer. Incubating purified GΔ228-Uve1p with DHU/A-37mer rather than DHU/G-37mer resulted in a 4-fold decrease in the amount of Uve1p-mediated cleavage products observed. To determine whether Uve1p cleaves the complementary strand of these substrates (i.e., U/A-37mer, DHU/A-37mer or U/G-37mer, DHU/G-37mer), we conducted similar experiments with these substrates except that the complementary strand was 3' end-labeled. No cleavage products were observed when these substrates were incubated with purified Uve1p protein preparations. Uve1p recognizes and cleaves uracil and DHU when they are placed opposite a G (U/G or DHU/G). However, when the lesions are placed in a situation where Watson-Crick hydrogen-bonding is maintained (U/A or DHU/A), Uve1p either fails to recognize the lesion completely (U/A) or the extent of cleavage is significantly decreased (DHU/G).

Uve1p recognizes and cleaves oligonucleotide substrates containing AP sites, uracil and DHU lesions. AP sites appear to be better substrates for Uve1p than uracil or DHU containing oligonucleotides; Uve1p cleaved AP sites at least 10 times more efficiently than uracil containing substrates and twice as efficiently as DHU containing substrates. However, they are all poorer substrates than UV-induced photoproducts. See Table 3 for a summary of the relative efficiency for cleavage by Uve1p on various substrates.

Additionally, the Uve1p preparations were incubated with the following substrates to determine if these lesions were capable of being cleaved by Uve1p: inosine and xanthine placed opposite a T or C (I/T-31mer, I/C-31mer and Xn/T-31mer, Xn/C-31mer), and 8-oxoguanine placed opposite all four bases (8-oxoG/G-37mer, 8-oxoG/A-37mer, 8-oxoG/T-37mer, 8-oxoG/C-37mer). No cleavage of either strand in these duplex substrates was observed.

As discussed hereinabove, because of substantial structural differences between CPDs and 6-4PPs, it was not obvious what features of damaged DNA Uve1p recognizes. One possibility is that Watson-Crick base pairing is disrupted for the 3' pyrimidines in both CPDs and 6-4PPs [Jing et al. (1998) *Nucl. Acids. Res.* 26:3845-3853], suggesting that Uve1p might target its activity to mispaired bases in duplex DNA. We therefore investigated the ability of purified GΔ228-Uve1p to cleave duplex oligonucleotides containing all possible combinations of single base mispairs embedded within the same flanking sequence context. For these studies, we utilized a collection of mismatch-containing oligonucleotides (series XY-31mer) which were designed so as to generate all possible mismatch combinations (Table 1B). Strands GX, AX, TX and CX were 3' end-labeled and then annealed to strands GY, AY, TY or CY prior to incubation with purified GΔ228-Uve1p. Reaction products were analyzed on DNA sequencing-type gels (See Examples). The ability of GΔ228-Uve1p to cleave all twelve possible mispair combinations is shown in Fig. 7A-7D. No DNA strand cleavage was observed for duplex substrates containing normal Watson-Crick G/C or A/T base pairs.

The sites of GΔ228-Uve1p-mediated mismatch-specific DNA cleavage were identified in each case by comparing the electrophoretic mobilities of the DNA strand scission products to those of a DNA sequencing ladder obtained by base-specific chemical cleavage. Arrows a, b, and c indicate the DNA strand scission products corresponding to cleavage by GΔ228-Uve1p immediately (position 0), one (position -1) or two (position -2) nucleotides 5' to the site of the mismatch, respectively (Fig. 7A-D). These sites of GΔ228-Uve1p-mediated endonucleolytic cleavage were confirmed in similar experiments employing 5' end-labeled GX, AX, TX and CX strands in the mismatch substrates. In addition, the non-truncated, full-length GFL-Uve1p (in crude cell extracts) recognized and cleaved *CX-AY-31mer in a manner identical to GΔ228-Uve1p (Fig. 11B). The preferred sites of cleavage and the efficiency with which each mismatch is recognized by GΔ228-Uve1p is variable and depends on the type of base mispair that is presented to the enzyme. Within the sequence context examined, GΔ228-Uve1p exhibited strong cleavage at *C/C (asterisk - labeled strand base), *C/A and *G/G sites, moderate cleavage at *G/A, *A/G and *T/G sites, and weak cleavage at *G/T, *A/A, *A/C, *C/T, *T/T and *T/C

sites. These differences in the extent of cleavage were reproducible and observed in three separate experiments. These results indicate that the GΔ228-Uve1p mismatch endonuclease activity has a preference for certain base mismatch combinations (e.g. *C/A) over others (e.g. *T/C). However, these experiments do not rule out an effect on cleavage by the sequence(s) flanking the mismatch.

Uve1p has been shown to incise DNA containing CPDs and 6-4PPs directly 5' to the photoproduct site generating products containing 3'-hydroxyl and 5'-phosphoryl groups [Bowman et al. (1994) *supra*]. We examined whether similar 3' and 5' termini were produced following Uve1p-mediated cleavage of base mismatch-containing substrates. DNA strand scission products generated by GΔ228-Uve1p cleavage of 3' end-labeled oligo *CX/AY-31mer (CX strand labeled, Table 1B) were further treated with calf intestinal phosphatase (CIP) which removes 5' terminal phosphoryl groups from substrate DNA. The major sites of Uve1p-mediated DNA cleavage relative to the base mispair site were found to be at positions 0 and -1 (Fig. 11A, lane 2). CIP treatment of these DNA cleavage products resulted in species that had retarded electrophoretic mobilities compared to non-CIP-treated DNA cleavage products, indicating a decrease in charge corresponding to removal of 5' terminal phosphoryl groups (Fig. 11A, lanes 2 and 3). In addition, GΔ228-Uve1p mismatch endonuclease-generated DNA cleavage products were resistant to phosphorylation by polynucleotide kinase, an expected result if the 5' termini already contain phosphoryl groups (Fig. 11A, lane 4). Electrophoretic mioibility shift analysis utilizing 5' end-labeled *CX/AY-31mer, terminal deoxyribonucleotidyl transferase (TdT), and α^{32}P-dideoxyATP (ddATP) resulted in addition of a single ddAMP to the 3' end of GΔ228-Uve1p-generated DNA cleavage products and indicates the presence of a 3'-hydroxyl terminus. These results show that the 3' and 5' termini of the products of GΔ228-Uve1p-mediated cleavage of substrates containing single base mismatches are identical to those generated following cleavage of substrates containing CPDs or 6-4PPs.

To verify that the Uve1p mismatch endonuclease activity observed was not the result of trace endonucleolytic contamination from the *S. cerevisiae* expression system and to determine

whether full length Uve1p was also capable of mismatch endonuclease activity, extracts from cells overexpressing GFL-Uve1p, GΔ228-Uve1p, and GST tag alone were tested for their abilities to cleave 5' end-labeled *CX/AY-31mer. Both GFL-Uve1p and GΔ228-Uve1p cleaved the base mismatch-containing substrate at positions 0, -1, and -2 (Fig. 11B). We also observed a weak 3' to 5' exonucleolytic activity associated with both crude GFL-Uve1p preparations and purified GΔ228-Uve1p which shortened the Uve1p-mediated cleavage products by one to three nucleotides (Fig. 11B, lanes 1 and 2). These shorter products are not due to additional cleavages by Uve1p mismatch endonuclease activity because they are not observed in identical experiments with 3' end-labeled substrates. Purified Δ228-Uve1p obtained following thrombin cleavage of the GST tag also possessed mismatch endonuclease activity. In contrast, no cleavage of mismatch-containing substrates was observed when extracts from cells transfected with vector expressing only the GST tag were tested. Thus, both GFL-Uve1p and its more stable, truncated version, GΔ228-Uve1p, both possess mismatch endonuclease activities.

GΔ228-Uve1p mismatch endonuclease and GΔ228-Uve1p UV photoproduct endonuclease share similar properties and compete for the same substrates. GΔ228-Uve1p requires divalent cations for activity and exhibits optimal activity against UV photoproducts in the presence of 10 mM $MgCl_2$ and 1 mM $MnCl_2$. Omission of divalent cations from the reaction buffer abolished GΔ228-Uve1p mismatch endonuclease activity on 5' end-labeled *CS/AY-31mer. The pH optimum for GΔ228-Uve1p mismatch endonuclease activity on this same substrate was found to be 6.5, which corresponds to the pH where optimal activity is observed against UV photoproducts.

To further confirm that the mismatch endonuclease activity was mediated by GΔ228-Uve1p, a substrate competition experiment was performed with CPD-30mer, a known Uve1p substrate which contains a centrally located UV photoproduct (CPD). Addition of increasing amounts of unlabeled CPD-30mer resulted in a significant, concentration-dependent decrease in GΔ228-Uve1p-mediated mismatch endonuclease activity against 3' end-labeled *CX/AY31mer (C/A mispair) (Fig. 12). In contrast, increasing amounts of the undamaged oligo GX/CY-31mer

(G/C base pair) had only a modest inhibitory effect, and inhibition did not increase with increasing amounts of added oligo, indicating a non-specific binding to Uve1p within this concentration range. In a similar experiment both unlabeled CPD-30mer and CX/AY-31mer (C/A mispair) were more potent inhibitors of 3' end-labeled *CX/AY-31mer cleavage compared to unlabeled GX/CY-31mer. The effective competition by CPD-30mer for mismatch endonuclease activity indicates that both base mismatch and UV photoproduct endonuclease activities are associated with GΔ228-Uve1p.

Uve1p incises only one strand of a duplex containing a base mismatch. Since Uve1p recognizes all possible base mismatch combinations, we determined whether the enzyme could incise both strands on the same molecule resulting in a DNA double strand break. An oligonucleotide (*CX/AY-41mer) was designed such that the base mispair was placed in the center of the oligonucleotide. GΔ228-Uve1p was incubated with 3' end-labeled *CS/AY-41mer under standard conditions, and the DNA strand scission products were analyzed on both non-denaturing and denaturing gels (Fig. 13A-13B). In the event that GΔ228-Uve1p created a DNA double strand break by incising 5' to the base mismatch site on the two complementary strands, the resulting products would possess an electrophoretic mobility similar to those created by the restriction enzyme DdeI (which cleaves adjacent to the mismatch) when analyzed on a non-denaturing polyacrylamide gel. In contrast, if GΔ228-Uve1p incises on either (but not both) complementary strands, then the resulting product would be a full-length duplex containing a single strand nick which would co-migrate with uncut duplex *CX/AY-41mer on a non-denaturing gel. Non-denaturing gel analysis of GΔ228-Uve1p-treated *CX/AY-41mer generated a product with an electrophoretic mobility identical to the untreated duplex with no products detected corresponding to those created by a double strand break (Fig. 13A). Denaturing gel analysis revealed a GΔ228-Uve1p-generated DNA strand scission product resulting from a single strand break of the labeled strand of either *CX/AY-41mer or CX/*GY-41mer. Together with the non-denaturing gel analysis, these results indicate that within the GΔ228-Uve1p substrate population, nicks occur on one or the other, but not both strands (Fig. 13B). These results show that GΔ228-Uve1p nicks only one of the two strands containing a base mismatch and that it does

not make double strand breaks in duplex DNA. Similarly, double strand breaks are not made in DNA molecules containing other structural distortions.

Without wishing to be bound by theory, it is believed that GΔ228-Uve1p possesses strand specificity directed towards the 3' terminus. Mismatched bases in duplex DNA are distinct from damaged DNA in the sense that both of the bases are usually undamaged per se, yet one is an inappropriate change in the nucleotide sequence and must be identified as such and removed. If Uve1p participates in MMR *in vivo*, how might it distinguish between the correct and incorrect bases in a mispair? One possibility is that proximity of the mispaired base to either the 3' or 5' terminus targets Uve1p mismatch endonuclease activity to a particular strand. For example, in DNA synthesis, chain growth proceeds from the 5' to the 3' terminus and newly-generated base misincorporations on the synthesized strand would be located in close proximity to the 3' terminus. Initiating the removal of such bases by a mismatch repair protein might involve association with a region of DNA in the vicinity of the 3' terminus, followed by targeting of the mispaired base located on that strand. To investigate this possibility, a series of 3' end-labeled oligonucleotides were generated that contained a C/A mispair located at various distances from the ends (Table 1B). The ability of GΔ228-Uve1p to incise the C-containing strand as a function of the distance of C (of the C/A mispair) from the 3' terminus was assessed by quantifying the GΔ228-Uve1p mismatch endonuclease-generated DNA stand scission products following denaturing gel analysis. A minimum level of mismatch cleavage was observed for C at a distance of 16 bp from the 3' terminus and gradually increased to a maximum for C at a distance of 16 bp from the 3' terminus. Closer placement (11 bp) of C to the 3' terminus resulted in a decrease in mismatch endonuclease activity with a complete loss of activity observed at a distance 6 bp from the 3' terminus. The mismatched base located on the strand in closest proximity to the 3' terminus is cleaved preferentially by GΔ228-Uve1p.

uve1 null mutants exhibit a mutator phenotype. We have examined the spontaneous mutation rate of *uve1::ura4*$^+$ disruption mutants as assayed by the ability to form colonies resistant to the toxic arginine analog L-canavanine. Uptake of L-canavanine in *S. pombe* is

mediated by an arginine permease encoded by the *can1*$^+$ gene [Fantes and Creanor (1984) *J. Gen. Microbiol.* 130:3265-3273]. Mutations in *can1*$^+$ eliminate the uptake of L-canavanine, and mutant cells are able to form colonies on medium supplemented with L-canavanine, whereas wild type cells cannot. We have compared the rate of spontaneous mutagenesis at the *can1*$^+$ locus in *uve1::ura4*$^+$ disruption mutants (Sp362) to both a negative control (wild type, 972) and a positive control, *pms1::ura4*$^+$ (see Example 11 hereinbelow). The *pms1* gene product is a homolog of *E. coli* MutL, and loss of *pms1* causes a strong mitotic mutator phenotype and increased postmeiotic segregation [Schar et al. (1997) *Genetics* 146:1275-1286].

To determine the relative sensitivity of each yeast strain to L-canavanine, 200 cells from mid-log phase cultures were plated onto PMALU^g plates supplemented with increasing concentrations of L-canavanine. Each of the strains was equally sensitive to L-canavanine. All strains were viable in the presence of lower concentrations of L-canavanine up to and including concentrations of 2.2 μg/ml, while concentrations higher than this were toxic to all strains. However, the colonies which grew in the presence of 2.2 μg/ml L-canavanine were smaller in diameter than the colonies which grew in the presence of lower concentrations.

The mean spontaneous mutation rate of each of the three strains was examined using fluctuation analyses. Single colonies grown on PMALU^g plates were used to inoculate liquid PMALU^g cultures which were grown to saturation. 10^7 cells were plated onto PMALU^g containing 75 μg/ml L-canavanine sulfate. The number of colonies on 24 plates for each strain was counted after 8 days incubation at 30°C. Both *uve1::ura4*$^+$ and *pms1::ura4*$^+$ strains showed an elevated number of resistant colonies compared to wild type. Additionally, the range of values for *uve1::ura4*$^+$ was broader and higher than for either wild type or *pms1::ura4*$^+$ and included two confluent plates scored as containing >5000 colonies. The mean rate of mutation was estimated using the method of the median [Lea and Coluson (1943) *J. Genet.* 49:264-284) using the median values. The calculated mutation rates are 1.5×10^{-7} (wild type), 9.7×10^{-7} (*uve1::ura4*$^+$), and 2.0×10^{-6} (*pms1::ura4*$^+$), indicating that *uve1::ura4*$^+$ mutants have a

spontaneous mutation rate approximately 6.5-fold greater than wild type and 2-fold lower than *pms1::ura4*$^+$. See Table 4 for a summary of results. Thus, loss of Uve1p confers a spontaneous mutator phenotype in *S. pombe*. In the mutation fluctuation analysis, a wide range of mutant colonies was observed for *uve1::ura4*$^+$ compared to *uve1::ura4*$^+$, suggesting that the pathways leading to mutation due to elimination of *uve1* and *pms1* are likely to be mechanistically different.

The finding that Uve1p recognizes all potential DNA base mispair combinations indicates that, in addition to its UV photoproduct cleavage activity, it is a diverse mismatch endonuclease with broad substrate specificity. In this regard, Uve1p is similar to *E. coli* endonuclease V [Yao and Kow (1994) *J. Biol. Chem.* 269:31390-31396], a *S. cerevisiae* and human "all-type" mismatch endonuclease [Chang and Lu (1991) *Nucl. Acids Res.* 19:4761-4766; Yeh et al. (1991) *J. Biol. Chem.* 266:6480-6484] and calf thymus topoisomerase I [Yeh et al. (1994) *J. Biol. Chem.* 269:15498-15504] which also recognize all potential base mismatch combinations. These enzymes incise DNA at each of the twelve base mispairs with variable efficiencies and either to the 5' (human all-type mismatch endonuclease) or 3' (*E. coli* endonuclease V) sides of a mismatch. Uve1p shows a preference for *C/C and *C/A mispairs, a property similar to the human all-type mismatch endonuclease [Yeh et al. (1991) *supra*]. In contrast, the strong preference of Uve1p for *G/G mispairs is a property which distinguishes Uve1p from all other mismatch endonucleases identified to date.

The biochemical properties of Uve1p-mediated mismatch cleavage and the spontaneous mutator phenotype displayed by *uve1* null mutants indicate that Uve1p is involved in MMR *in vivo*. The preference for making incisions on the strand harboring the mispaired base nearest to the 3' terminus reflects a discrimination strategy that might specifically target newly misincorporated bases during replication. Uve1p-generated incision 5' to the base mismatch site could be followed by a 5' to 3' exonuclease activity such as that mediated by *S. pombe* exonuclease I [Szankasi and Smith (1995) *Science* 267:1166-1169] or the FEN-1 homolog Rad2p

[Alleva and Doetsch (1998) *Nucl. Acids Res.* 26:3645-3650] followed by resynthesis and ligation.

S. pombe possesses at least two distinct mismatch repair systems and whether Uve1p mediates a role in either of these or represents a third, novel pathway is not known at present. The proposed major pathway does not recognize C/C mismatches and has relatively long (approximately 100 nt) repair tracts [Schar and Kohli (1993) *Genetics* 133:825-835]. Uve1p is thought to participate in a relatively short patch repair process which utilizes Rad2p (a FEN-1 homolog) DNA polymerase δ, DNA ligase and accessory factors [Alleva et al. (1998) *Nucl. Acids Res.* 26:3645-3650]. Based on these properties, it is unlikely that Uve1p is involved in a long tract mismatch repair system. The second, presumably less frequently utilized, (alternative) pathway recognizes all potential base mismatch combinations and has a repair tract length of about 10 nucleotides [Schar and Kohli (1993) *supra*]. These features of the alternative mismatch repair pathway are consistent with the repair properties of Uve1p based on recognition of C/C mismatches and short repair patch.

Unlike in repair of UV photoproducts, it is not clear in mismatch repair which base represents the nucleotide that needs to be removed. This can be explained by our finding that Uve1p prefers a mispaired base located near the 3' terminus of a duplex, which is consistent with Uve1p mediating mismatch repair for either leading or lagging strand synthesis during DNA replication. The preference for making incisions on the strand of the base nearest to the 3' terminus suggests a discrimination strategy to specifically target newly synthesized misincorporated bases. On the other hand, G/G, C/C mismatches are not frequently occurring base misincorporations encountered during replication, although they are among the most efficiently cleaved by Uve1p. A second role for Uve1p is in the correction of mismatched bases formed as a result of homologous recombination events where G/G and C/C mismatches would be expected to occur. A third role for Uve1p is in the repair of base bulges and loops generated as a result of primer-template misalignments during replication. Preliminary studies show that Uve1p mediates strand cleavage 5' to small bulges.

The present inventors have characterized the ability of Uve1p to recognize and incise duplex DNA containing mismatches, IDLs and hairpins. In addition, a determinant of the strand specificity for Uve1p-mediated DNA cleavage has been examined. Evidence that the 3'-terminus of the strand containing a mismatch is involved in directing the strand specificity of Uve1p is presented, and the implications for such strand discrimination are discussed.

What is the structural basis for lesion recognition by Uve1p? Previous studies with Uve1p have focused exclusively on its role in the repair of UV light-induced DNA damage, resulting in the notion that this enzyme functions in the repair of UV photoproducts exclusively, hence the prior name UVDE (UV damage endonuclease), now Uve1p. The results of this study clearly indicate a much broader involvement of Uve1p in *S. pombe* DNA repair and show that many other types of DNA lesions are recognized by this versatile repair protein. For example, we have recently found that Uve1p recognizes and incises DNA substrates containing uracil residues resulting from cytosine deamination, dihydrouracil, cisplatin-induced adducts as well as small base bulges. The molecular basis for substrate recognition by Uve1p is not obvious, but without wishing to be bound by theory, it is believed to be due in part to disruption of normal Watson-Crick base pairing and the corresponding changes expected in the electronic characteristics of the major and minor grooves of B-DNA.

Besides initiating repair of DNA containing UV damage including CPDs and 6-4PPa, UVDE and the truncated UVDE polypeptide of the present invention (Δ228-UVDE and/or GST-Δ228-UVDE) also initiate repair via cleavage of DNA duplexes containing the following base pair mismatches: C/A; G/A; G/G; A/A; and C/T. These experiments were conducted with GST-Δ228-UVDE. We also confirmed that the C/A mismatch is cleaved by Δ228-UVDE; it should also recognize the others. In addition, both GST-Δ228-UVDE and Δ228-UVDE recognize and cleave an oligonucleotides containing a GG-platinum diadduct formed by the antitumor agent cis-dichlorodiammineplatinum (II) (also known as cisplatin). Thus the substrate specificity range for UVDE is much broader than originally thought. Recognition of the truncated UVDE

polypeptide to initiate mismatch repair was made possible due to the increased stability of the presently exemplified truncated UVDE polypeptide in substantially purified form.

Herein we describe the ability of Uve1p to recognize and process IDL structures in duplex DNA as well as the determinants of the strand specificity of Uve1p. The ability of GΔ228-Uve1p to process DNA duplexes containing IDL and hairpin structures was investigated with a series of oligonucleotide substrates containing loops of 2-8 nucleotides in length or an 8 bp hairpin structure (Table 17A-17B). The DNA sequence and structures of the substrates are shown in Table 17A-17B. Strands I0, I2, I4, I6, I8 or HP8 were 3'-end-labeled and annealed to strand D0. This annealing reaction formed duplexes ID0, ID2, ID4, ID6, ID8 and HPD8, with 0, 2, 4, 6, 8 nt loops or an 8 bp hairpin, respectively. The substrates were treated with GΔ228-Uve1p (Figs. 14A-14C, even lanes) or with buffer alone (Figs. 14A-14C, odd lanes). No DNA strand cleavage was observed for the normal homoduplex substrate ID0 (Fig. 14A, lane 2). In contrast, GΔ228-Uve1p cleaves heteroduplexes containing insertions of 2 or 4 unpaired nucleotides (Fig. 14A, lanes 4 and 6). However, the enzyme did not cleave duplexes ID6 or ID8 containing larger loops of 6 and 8 nucleotides, respectively (Fig. 14C, compare lanes 2, 4 and 6).

The results shown in Figs. 14A-14C indicate that GΔ228-Uve1p produces several cleavage products from the strand containing a 2 or 4 nt unpaired loop. The sites of cleavage correspond to endonucleolytic cleavage of duplexes ID2 and ID4 at a distance of 1 (position a), 15 (position b) and 16 (position c) nucleotides 5' to the IDL (Fig. 14A, lanes 4 and 6). The major site of Uve1p-mediated cleavage on duplex ID2 was observed to be at positions b, and c, whereas the major site of cleavage on duplex ID4 was observed to be at position a. The cleavages at positions a, b and c were also observed when the DNA substrate was labeled at the 5'-terminus of the strand containing the loop (data not shown), which confirms that Uve1p cleaves endonucleolytically at these sites. Uve1p did not cleave DNA containing a base insertion within the context of a small hairpin structure (Fig. 14B, lane 2).

Uve1p has been shown to exhibit a wide substrate specificity, including bipyrimidine UV photoproducts, base mispairs, abasic sites, and platinum GG diadducts in duplex DNA [Kaur et al. (1999) *Mol. Cell. Biol.* 19:4703-4710; Avery et al. (1999) *Nucleic Acids Res.* 27(11):2256-2264; Kanno et al. (1999) *Nucleic Acids Res.* 27:3096-3103]. For each of these lesions, GΔ228-Uve1p cleaves endonucleolytically immediately, one or two nt 5' to the site of base damage. The cleavage events at positions b and c (Fig. 14A) located 15 and 16 nt 5' to the site of the loop of substrates ID2 or ID4 have been observed only with these types of DNA substrates containing small base insertions. Because cleavage events at similar distances 5' to the site of DNA damage were not observed in earlier studies, it seemed possible that the observed distal cleavage events were the result of a sequence-specific structure formed by the particular duplex used in this experiment. Therefore, the generality of this cleavage pattern was tested using IDL substrates within a different DNA sequence context.

DNA substrates were constructed by annealing oligonucleotides Lo0, Lo2 and Lo4 to strand Bot to generate duplexes LD0, LD2 and LD4 with loops of 0, 2 and 4 nucleotides, respectively (Table 17B). The strands which form a loop in the duplex (LD0, LD2 and LD4) were either labeled on the 3' (Fig. 15A) or the 5' (Fig. 15B) end. These substrates were incubated with purified GΔ228-Uve1p or with buffer, and the reaction products were analyzed on a 20% denaturing polyacrylamide gel (15A-15B). GΔ228-Uve1p cleaved duplexes LD2 and LD4 with base insertions of 2 or 4 nucleotides. However, as observed previously with the substrates ID6 and ID8, DNA substrates with 6 (LD6) or 8 (LD8) nt insertions were not cleaved by Uve1p (Fig. 14C and data not shown). These results indicate that GΔ228-Uve1p recognizes and cleaves duplex DNA with loops of 2 or 4 nucleotides but not IDL structures of 6 or 8 nucleotide insertions or certain hairpin structures. The extent of GΔ228-Uve1p-mediated cleavage observed with IDL substrates containing 2 and 4 nt insertions was approximately the same as that previously observed for a duplex DNA substrate containing a C/A mispair [Kaur et al. (1999) *supra*]. Furthermore, for each of these IDL containing duplexes, no cleavage of the opposite strand was observed.

To further investigate the involvement of Uve1p in the recognition and cleavage of substrates containing short IDL structures, antibody inhibition experiments were conducted. Rabbit polyclonal antibodies specific for Δ228-Uve1p were generated. GΔ228pUve1p was preincubated with 40 to 160 μg of pre-immune or immune serum, and endonuclease assays with 3'-end-labeled substrate ID2 were carried out under standard reaction conditions. Preincubation of GΔ228-Uve1p with this antibody significantly decreases GΔ228-Uve1p-mediated cleavage of substrate ID2 in a concentration-dependent manner (Fig. 16, lanes 2-5). In contrast, preincubation with pre-immune serum did not inhibit the GΔ228-Uve1p-mediated endonuclease at any concentration tested (Fig. 16, lanes 6-9). Western blot analysis of the purified GΔ228-Uve1p with the immune serum produced a single band, indicating that the antibody does not cross-react with any trace contaminating proteins in the purified GΔ228-Uve1p preparation used in these experiments. The specific inhibition of the IDL cleavage activity of GΔ228-Uve1p by the antibody indicates that the observed cleavage of the IDL containing substrates was associated with GΔ228-Uve1p.

We have demonstrated that Uve1p recognizes and processes UV photoproducts and all twelve base mispair combinations in a similar manner [Kaur et al. (1998) *Biochemistry* 37:11599-11604; Kaur et al. (1999) *supra*; Avery et al. (1999) *supra*]. We have further demonstrated that Uve1p is also capable of recognizing small IDL structures. We examined the features of known Uve1p substrates which confer specificity with respect to DNA strand cleavage preference. The substrate specificity of Uve1p *in vitro* indicates its usefulness in repair of mismatches or IDL structures *in vivo*. It was important to determine how the enzyme distinguishes between the "correct" and "incorrect" base in a mispair. One possibility is that the proximity of one of the mispaired bases to either the 3' or the 5' terminus might direct strand specificity for cleavage. For example, *E. coli* endonuclease V is a mismatch endonuclease that displays strong strand specificity, preferring to cleave the strand containing the mispaired base closest to the 5'-terminus [Yao and Kow (1994) *J. Biol. Chem.* 269:31390-31396]. We tested the possibility that the strand specificity of GΔ228-Uve1p is affected by the nature of the terminus in closest proximity to the mispair.

A series of oligonucleotides (41-mers) which form DNA duplexes with a single C/A mispair at different positions within the sequence and at different distances from the termini of the linear duplex DNA molecule were synthesized (Table 18). The C-containing strand was 3'-end labeled and the relative cleavage activity of GΔ228-Uve1p on this strand was quantified (Fig. 17A). DNA cleavage was relatively inefficient at the C on this strand when located 37 nt from the 3' terminus, gradually increased to a maximum when located 16 nt from the 3'-terminus, and was undetectable when located 6 nt from the 3'-terminus. A similar result was obtained when the A-containing strand of the C/A mispair was analyzed (Fig. 17B). These results indicate that the cleavage efficiency of Uve1p at a C/A mismatch is sensitive to the proximity of the mispaired base to the 3' terminus. However, in this experiment, the flanking DNA sequence context of the C/A mispair varies at different positions within the DNA duplex, which also influences the cleavage efficiency of Uve1p.

To gain further insight into the strand preferences of Uve1p-mediated DNA cleavage, four duplex DNA substrates (AB, AC, AD, and AE) were designed to test the cleavage efficiency of Uve1p at a C/C mismatch within the same sequence context but at different distances from 3' and 5' termini (Table 18). The length and sequence of the "A" strand of these duplexes was identical in all cases, and the length of the complementary strand (B, C, D, or E) was varied. The cleavage efficiency of GΔ228-Uve1p on strands B, C, D, and E in the substrates AB, AC, AD, or AE was quantified (Fig. 17C). Substrates AB and AC, in which the C/C mispair was 25 nt from the 3'-terminus were cleaved by GΔ228-Uve1p with a similar efficiency. In contrast, the efficiency of cleavage of substrates AD and AE in which the C/C mispair was 40 nt from the 3' terminus was about 2-fold lower. Moreover, the efficiency of cleavage of the duplex substrate appeared to be independent of the distance of the mismatch from 5'-terminus (compare AB with AC and AD with AE). These results are consistent with sensitivity of cleavage of a mispaired base by GΔ228-Uve1p to its proximity to the 3'-terminus of that strand.

Repetitive sequences in DNA are capable of forming IDL structures during replication, which can lead to insertion or deletion mutations [Petruska et al. (1998) *supra*; Pearson et al.

(1998) *supra*]. MMR systems in both prokaryotes and eukaryotes are utilized for the repair of such IDL structures [Modrich (1991) *supra*; Kolodoner, R. (1996) *supra*; Strand et al. (1993) *supra*]. We have demonstrated that Uve1p incises small IDL structures in DNA, indicating that it helps maintain the stability of repetitive sequences in the genome. Our results also show that Uve1p cleaves DNA 15-16 nucleotides 5' to the IDL structure (Figs. 14A-14C and 15A-15B). The reasons for such a distal cleavage event are unknown. Without wishing to be bound by any particular theory, we believe that Uve1p causes a distortion in the DNA when it binds to a mismatch and that this distortion leads to the distal cleavage event. In this case, cleavage at the distal site results from a conformation of Uve1p and DNA unique to the complex formed by Uve1p at an IDL.

The substrate specificity of Uve1p, including IDL structures as well as base mispairs, is not a unique property of Uve1p and its truncated derivatives. *E. coli* endonuclease V, spinach nuclease SP, and T4 endonuclease VII also share these properties in that they recognize and cleave DNA containing base mispairs and small IDLs [Mizuuchi et al. (1982) *Cell* 29:357-365; Golz et al. (1996) *DNA Res.* 2:277-284; Greger and Kemper (1998) *Nucleic Acids Res.* 26(19):4432-4438]. However, these enzymes also differ from each other with respect to the sites and extent of cleavage in proximity to the DNA distortion as well as their recognition of other types of DNA damages and other types of DNA structures. In addition, the physical characteristics and environment in which optimal activity varies considerably from enzyme to enzyme.

Uve1p does not recognize hairpin type structures. In this respect, Uve1p is distinct from *E. coli* endonuclease V, but similar to *S. cerevisiae* insertion mismatch recognition activity [Miret et al. (1996) *Nucleic Acids Res.* 24(4):721-729]. IDL structures that form hairpins are considered to be poor substrates for repair [Nag et al. (1989) *Nature* 340(6231):318-320] and demonstrate a low rate of repair *in vivo* in *S. cerevisiae* [Moore et al. (1999) *Proc. Natl. Acad. Sci. USA* 96(4):1504-1509]. The recombinant GST tagged, truncated version of Uve1p has been

utilized in all these experiments. Without wishing to be bound by theory, we believe it reflects the substrate specificity of the native Uve1p enzyme *in vivo*.

In order to contribute to mismatch repair *in vivo* in a manner that maintains genomic stability, Uve1p requires a mechanism for strand discrimination. Our results indicate that during mismatch recognition and cleavage, the strand preference of Uve1p is determined by the proximity of a mispaired base to the 3'-terminus. This strand selection mechanism is believed to reflect the fact during DNA replication newly generated base misincorporations in the lagging strand are close to the DNA 3'-terminus associated with the replication fork. In this way, repair of mispair and IDL structures by Uve1p contribute to genomic stability *in vivo*.

Recent studies indicate that DNA repair pathways for damaged DNA bases are also in some cases involved in mismatch repair [Sancar, A. (1999) *supra*; Oleykowski et al. (1999) *Biochemistry* 38:2200-2205]. Rad1p, a nucleotide excision repair protein of *S. cerevisiae*, is considered important for the repair of DNA loops [Kirkpatrick and Petes (1997) *Nature* 387:929-931]. Biochemical as well as genetic evidence indicates that the Rad1p/Rad10p endonuclease is absolutely required for nucleotide excision repair of UV damaged DNA and that it also functions in genetic recombination [Davies et al. (1995) *J. Biol. Chem.* 270:24638-24641; Habraken et al. (1994) *Nature* 371:531-534; Bardwell et al. (1994) *Science* 265:2082-2085]. *S. pombe* Rad16p and Swi10p, are the homologs of the yeast Rad1p and Rad10p. Mutations in these two proteins reduce the frequency of mating-type switching and also confer a radiation-sensitive phenotype [Schmidt et al. (1989) *Curr. Genet.* 16:89-94; Carr et al. (1994) *Mol. Cell. Biol.* 14:2029-2040; Hang et al. (1996) *Gene* 170(1):113-117]. There is also evidence indicating that mismatch repair proteins recognize various types of DNA adducts [Marra and Schar (1999) *Biochem. J.* 338:1-13]. Thus, Uve1p participates in the repair of UV-induced DNA damage as well as DNA replication-associated mismatch or IDL lesions of five or fewer nucleotides in the loop. We further believe Uve1p acts in concert with both DNA excision repair and mismatch repair pathways.

Skin cancers associated with sunlight exposure are the most common worldwide human cancers. The primary DNA damage from exposure to sunlight are 6-4 PPs and CPDs. Since UVDE can augment cells defective in DNA repair, the stable truncated UVDE fragments of the present invention will be valuable therapeutic agents for correcting DNA repair defects in sunlight-sensitive and skin cancer-prone individuals, for example individuals with the genetic disease xeroderma pigmentosum. Additionally, GST-Δ228-UVDE and Δ228-UVDE can be used as protective agents against sunlight-induced skin damage in normal individuals because they can augment the existing DNA repair levels of CPDs and 6-4 Pps and other DNA damage.

Homologs of the *S. pombe* UVDE protein have been identified by BLAST searching of sequence database (Genbank, TIGR) using the UVDE amino acid sequence: *N. crassa* (Genbank Accession No. BAA 74539), *B. subtilis* (Genbank Accession No. 249782), human (Genbank Accession No. AF 114784.1, methyl-CpG binding endonuclease) and a *Deinococcus radiodurans* sequence located from the TIGR database. The amino acid sequences of these proteins are given in SEQ ID NO:36 (*N. crassa*), SEQ ID NO:37 (*B. subtilis*), SEQ ID NO:38 (*Homo sapiens*) and SEQ ID NO:39 (*D. radiodurans*). The *D. radiodurans* coding sequence can be generated using the genetic code and codon choice according to the recombinant host in which the protein is to be expressed, or the natural coding sequence can be found on the TIGR database, *D. radiodurans* genomic sequence in the region between bp 54823 and 60981. Additional homologs of the *S. pombe* UVDE include the UV damage enzyme of *Bacillus anthracis*; *Halobacterium* sp., disclosed in Genbank Accession No. AAC 82899; *Methanococcus jannaschii,* disclosed in Genbank Accession No. 057597; and *Thermotoga maritima*, disclosed in Genbank Accession No. AE001740. These homologs were identified using Blast or FastA on the NCBI or TIGR websites. A Uve1p consensus sequence has been derived using the vector NTI AlignX program. This consensus sequence spans amino acids 308-465 of the C-terminal region of the *S. pombe* Uve1p. This region shows significant sequence similarity to portions of the *B. subtilis* and *N. crassa* Uve1p equivalents. The alignments for this region are shown in Table 19. The partial amino acid sequences of the *Bacillus anthracis*, *Halobacterium* sp., *Methanococcus jannaschii*, and *Thermotoga maritima* are provided in SEQ ID Nos: 72-75. The

consensus sequence is given in SEQ ID NO:76, and the sequence alignment and consensus sequence are illustrated in Table 24.

The regions of the *S. pombe* UVDE protein which are most conserved in the foregoing homologs are amino acids 474-489, 535-553, 578-611, 648-667, 711-737 and 759-775 of SEQ ID NO:2.

The stable truncated UVDE derivatives of the present invention are useful to treat or prevent diseases caused by cyclobutane pyrimidine dimers or (6-4) photoproducts or DNA mismatch, abasic sites or other distortions in the structure of double stranded DNA through the application of skin creams which can deliver GST-Δ228-UVDE and Δ228-UVDE to the appropriate living cells or via other routes of administration with compositions suitable for the route of administration, as is well understood in the pharmaceutical formulation art. GST-Δ228-UVDE or Δ228-UVDE can be incorporated into liposomes, and the liposomes can be applied to the surface of the skin, whereby the encapsulated GST-Δ228-UVDE and Δ228-UVDE products traverse the skin's stratum corneum outer membrane and are delivered into the interior of living skin cells. Liposomes can be prepared using techniques known to those skilled in the art. A preferred liposome is a liposome which is pH sensitive (facilitates uptake into cells). Preparation of pH sensitive liposomes is described in U.S. Pat. No. 5,643,599, issued to Kyung-Dall et al.; and 4,925,661 issued to Huang. The GST-Δ228-UVDE and Δ228-UVDE polypeptides can be entrapped within the liposomes using any of the procedures well known to those skilled in the art. See, e.g., the Examples and U.S. Pat. Nos. 4,863,874 issued to Wassef et al.; 4,921,757 issued to Wheatley et al.; 5,225,212 issued to Martin et al.; and/or 5,190,762 issued to Yarosh.

The concentration of liposomes necessary for topical administration can be determined by measuring the biological effect of GST-Δ228-UVDE and Δ228-UVDE, encapsulated in liposomes, on cultured target skin cells. Once inside the skin cell, GST-Δ228-UVDE or Δ228-UVDE repairs CPDs or 6-4 Pps in damaged DNA molecules and increases cell survival of those cells damaged by exposure to ultraviolet light.

Polyclonal or monoclonal antibodies specific to GST-Δ228-UVDE and Δ228-UVDE allow the quantitation of GST-Δ228-UVDE and Δ228-UVDE entrapped into liposomes. GST-Δ228-UVDE and Δ228-UVDE antibodies also allow tracing of the truncated UVDE polypeptides into skin cells.

Standard techniques for cloning, DNA isolation, amplification and purification, for enzymatic reactions involving DNA ligase, DNA polymerase, restriction endonucleases and the like, and various separation techniques are those known and commonly employed by those skilled in the art. A number of standard techniques are described in Sambrook et al. (1989) *Molecular Cloning*, Second Edition. Cold Spring Harbor Laboratory, Plainview, New York; Maniatis et al. (1982) *Molecular Cloning*, Cold Spring Harbor Laboratory, Plainview, New York; Wu (ed.) (1993) *Meth. Enzymol.* Part I; Wu (ed.) (1979) *Meth Enzymol.* 65; Miller (ed.) (1972) *Experiments in Molecular Genetics*, Cold Spring Harbor Laboratory, Cold Spring Harbor, New York; Old Primrose (1981) *Principles of Gene Manipulation*, University of California Press, Berkeley; Schleif and Wensink (1982) *Practical Methods in Molecular Biology*; Glover (ed.) (1985) *Nucleic Acid Hybridization*, IRL Press, Oxford, UK; and Setlow and Hollaender (1979) *Genetic Engineering*: *Principles and Methods*, Vols. 1-4, Plenum Press, New York. Abbreviations and nomenclature, where employed, are deemed standard in the field and commonly used in professional journals such as those cited herein.

Each reference cited in the present application is incorporated by reference herein to the extent that it is not inconsistent with the present disclosure.

The following examples are provided for illustrative purposes, and are not intended to limit the scope of the invention as claimed herein. Any variations in the exemplified articles which occur to the skilled artisan are intended to fall within the scope of the present invention.

EXAMPLES

Example 1. Strains, enzymes, plasmids and genes.

E. coli Top10 (Invitrogen Corp., San Diego, CA) was used for subcloning and plasmid propagation. *S. cerevisiae* strain DY150 used for protein expression and the *S. cerevisiae* expression vector pYEX4T-1 were purchased from Clontech (Palo Alto, CA).

S. pombe strains used in this study include 972, h^{-s} [Leupold, U. (1970) *Meth. Cell Physiol.* 4:169-177]; PRS301, h^{-s} *pms1::ura4*$^{+}$ [Schar et al. (1993) *Genetics* 146:1275-1286]; SP30, h^{-s} *ade6-210 leu-32 ura4-D18* [Davey et al. (1998) *Mol. Cell. Biol.* 18:2721-2728]. Sp362 (h^{-s} *ade6-210 leu1-32 ura4-D18 uve1::ura4*$^{+}$) was constructed by transforming Sp30 with a linearized, genomic *uve1*$^{+}$ fragment derived from pgUV2 [Davey et al. (1997) *Nucl. Acids Res.* 25:1005-1008] in which nucleotides 215 (*EcoRI*) to 1045 (*ClaI*) of *uve1*$^{+}$ were replaced with the *ura4+* gene. Extracts of Sp362 contained no detectable Uve1p activity against CPD-30mer. Cultures were grown in pombe minimal medium (PM) [Leupold (1970) *supra*] with 3.75 g/l glutamate replacing ammonium chloride as the nitrogen source [Fantes and Creanor (1984) *J. Gen. Microbiol.* 130:3265-3273], and were supplemented with 150 mg/l of each adenine, leucine and uracil (PMALU^{g}). Solid media was prepared by addition of 20 g/l agar. L-canavanine sulfate was sterilized prior to addition to the medium.

Purified mismatch repair endonuclease, *E. coli* endonuclease V [Yao and Kow (1997) *J. Biol. Chem.* 272:30774-30779] was a gift from Yoke Wah Kow (Atlanta, GA).

Example 2. Amplification of the *uvde* (*uve1*) gene from *S. pombe*.

A cDNA library purchased from ATCC was amplified by PCR, using the sense primer: 5'-TGAGGATCCAATCGTTTTCATTTTTTAATGCTTAGG-3' (SEQ ID NO:9) and the antisense primer: 5'-GGCCATGGTTATTTTTCATCCTC-3' (SEQ ID NO:10). The gene fragment of interest was amplified in the following manner. Four hundred nanograms of template DNA (*S. pombe* cDNA library) was incubated with the upstream and downstream

primers (300nM) in the presence of Pwo DNA polymerase (Boehringer Mannheim, Indianapolis, IN) in 10 mM Tris-HCl (pH 8.85), 25 mM KCl, 5 mM $(NH_4)_2SO_4$, 2mM $MgSO_4$ and 200 μM of dNTPs. The DNA was initially denatured at 94°C for 2 min. Three cycles of denaturation at 94°C for 15 sec, annealing at 45°C for 30 sec and primer extension at 72°C for 2 min were followed by twenty cycles using 50°C as the annealing temperature. All other incubation times and temperatures remained the same. The amplification was completed by a final primer extension at 72°C for 7 min.

Example 3. Amplification of the Δ228-UVDE gene-encoding fragment from *S. pombe*.

PCR was used to produce a truncated DNA fragment of the full-length *S. pombe uvde* gene which encodes a protein product containing a deletion of 228aa from the N-terminal portion of the full-length *S. pombe* UVDE protein. The following primers were used in the PCR reaction to amplify the gene fragment which encodes Δ228-UVDE: sense primer 5'-AATGGGATCCGATGATCATGCTCCACGA-3' (SEQ ID NO:11) and the antisense primer 5'-GGGATCCTTATTTTCATCCTCTTCTAC-3' (SEQ ID NO:12). PCR conditions were as described in Example 2.

Example 4. Purification of Δ228-UVDE and full-length UVDE.

The amplified UVDE gene coding fragments were cloned into the *BamHI* and *SmaI* restriction sites of pYEX 4T-1. The Δ228-UVDE gene coding fragments were cloned into the *BamHI* restriction site of pYEX4T-1 (Clontech, Palo Alto, CA). In the pYEX4T-1 vector, the coding region of both the proteins is expressed in frame with a glutathione-S-transferase (GST) leader sequence to generate a fusion protein of GST linked to the N-terminus of UVDE which is under the control of the CUP1 promoter [Ward et al. (1994) *Yeast* 10:441-449]. The subcloned plasmids were checked for orientation by restriction analysis and were then transformed into *S. cerevisiae*, DY150 cells, using the alkali cation method [Ito et al. (1983) *supra*]. A single positive clone was picked and grown at 30° C until mid log phase. Cultures in mid log phase were induced with 0.5 mM $CuSO_4$. Cells (500 mL) were harvested 1 or 2 hr after induction and lysed with glass beads in 50 mM Tris (pH 7.5), 100 mM EDTA, 50 mM NaCl, 10 mM β-

mercaptoethanol, 5% glycerol in the presence of 10 ng/mL pepstatin, 3 nM leupeptin, 14.5 mM benzamidine, and 0.4 mg/mL aprotinin. The cell lysate was then dialyzed overnight in buffer minus EDTA. The whole cell homogenate was separated into soluble and insoluble fractions by centrifugation at 45,000 X g for 20 min. The soluble proteins (120 mg) were applied to a 2 mL glutathione-Sepharose-affinity column (Pharmacia, Piscataway, NJ). All purification steps were carried out at 4° C and are similar to the strategies employed for the purification of other types of GST-tagged proteins [Ward et al. (1994) *Yeast* 10:441-449; Harper and Speicher (1997) In: *Current Protocols in Protein Sci.* (Coligan, J. et al., Eds), John and Wiley & Sons, pp. 6.6.1-6.6.21). Unbound proteins were removed by washing with 30 mL phosphate-buffered saline (pH 7.4), 5 mM EDTA, 0.15 mM PMSF. GST-Δ228-UVDE was eluted (100-200 μL fractions) with 10 mM glutathione in 50 mM Tris (pH 7.4) or cleaved on the column with excess of thrombin as previously described [Harper and Speicher (1997) *supra*] to generate Δ228-UVDE without the GST tag. SDS-PAGE analysis of flow-through, wash, elution, and thrombin cleavage fractions indicated the extent of purification or GST tag removal via thrombin cleavage (Fig. 1A-1B). Typically, the GΔ228Uve1p was nearly homogeneous. Purified proteins were stored at -80°C in 10% glycerol.

Example 5. GST preparation.

S. cerevisiae (DY150) cells were transformed with the pYex4T-1 expression vector without any insert (i.e., expressing gluthathione-S-transferase [GST] alone). These cultures were induced with $CuSO_4$ and cell lysates were prepared as described for the Uve1p proteins. Purified recombinant GST was affinity-purified on a gluthathione sepharose column in an identical manner to GΔ228-Uve1p (see above) and was included in all of the assays performed in this study as a control for trace amounts of potential contaminating endonucleases in the Uve1p protein preparations.

Example 6. UVDE activity assay and optimization of reaction conditions.

Crude and purified full-length UVDE, GST-Δ228-UVDE and Δ228-UVDE were tested for activity on an oligodeoxynucleotide substrate (CPD-30mer) containing a single cis-syn

cyclobutane pyrimidine dimer embedded near the center of the sequence. The sequence of the CPD-containing strand is: 5'-CATGCCTGCACGAAT^TAAGCAATTCGTAAT-3' (SEQ ID NO:13) . The CPD-containing DNA molecule was synthesized as described by Smith and Taylor (1993) *J. Biol. Chem.* 268:11143-11151. The CPD-30mer was 5' end labeled with [γ-^{32}P]ATP (Amersham, 3000 Ci/mmol) using polynucleotide kinase [Tabor (1985) *vide infra*]. For UVDE reactions with end labeled CPD-30mer, approximately 10 fmol of 5' end labeled CPD 30-mer was incubated with 5-100 ng of Δ228-UVDE or GST-Δ228-UVDE, in 200 mM Hepes (pH 6.5), 10 mM $MgCl_2$, 1 mM $MnCl_2$, 150 mM NaCl for 15 min at 37° C 10-20 μL reaction volume). The reaction products were analyzed on 20% denaturing (7 M urea) polyacrylamide gels (DNA sequencing gels) as previously described (Doetsch, et al., 1985). The DNA species corresponding to the uncleaved CPD-30mer and cleavage product (14-mer) were analyzed and quantified by phosphorimager analysis (Molecular Dynamics Model 445SI) and autoradiography.

In other experiments, reactions with various Uve1p preparations were carried out in a total volume of 20 μL, and contained reaction buffer (20 mM Hepes, pH 6.5, 100 mM NaCl, 10 mM $MgCl_2$ and 1 mM $MnCl_2$) and end-labeled oligonucleotide substrate (10-30 fmol). The substrate/buffer mix was incubated for 20 min at 37°C with Uve1p. In the case of G-Uve1p and GΔ228-Uve1p, crude cell lysates (5 μg of protein) were used for all assays. Fifty ng of affinity-purified GΔ228-Uve1p (0.75 pmol) and Δ228-Uve1p (1.2 pmol) were incubated with all of the UV-induced photoproducts. For all other assays 2 μg of affinity-purified GΔ228-Uve1p (30 pmol) and Δ228-Uve1p (48 pmol) were incubated with the substrates. Two μg of affinity-purified recombinant GST (72 pmol) was incubated with each substrate under Δ228-Uve1p optimum reaction conditions to control for potential contaminating nuclease activities which may be present in the Uve1p preparations and to determine the specificity of the Uve1p cleavage reaction. DNA repair proteins (*E. coli* exonuclease III, *E. coli* endonucleases III and IV, *E. coli* uracil DNA glycosylase and *S. cerevisiae* endonuclease III-like glycosylase [Ntg]) specific for each oligonucleotide substrate were also incubated with these substrates under their individual optimum reaction conditions, as a means to determine the specific DNA cleavage sites of Uve1p.

The reaction products were analyzed on 20% denaturing (7M urea) polyacrylamide gels (DNA sequencing-type gels) as described previously [Doetsch et al. (1985) *Nucl. Acids Res.* 13:3285-3304]. The DNA bands corresponding to the cleaved and uncleaved substrate were analyzed and quantified by phosphorimager analysis (Molecular Dynamics Model 445SI) and autoradiography.

Example 7. Oligonucleotides containing DNA damage.

The DNA damage-containing oligonucleotides used as substrates in this study are presented in Table 1A. The structure of each damaged lesion is presented in Figure 1. The 30-mer cs-CPD-containing oligonucleotide (cs-CPD-30mer) was prepared as described previously [Smith, C.A. (1993) *J. Biol. Chem.* 268:11143-11151]. The 49-mer oligonucleotides containing a cs-CPD (cs-CPD-49mer), a ts I-CPD (tsI-CPD-49mer), a ts II-CPD (tsII-CPD-49mer), a 6-4PP (6-4PP-49mer) and a Dewar isomer (Dewar-49mer) were synthesized as described previously [Smith and Taylor (1993) *J. Biol. Chem.* 268:11143-11151]. The oligonucleotide containing a platinum-DNA GG diadduct (Pt-GG-32mer) and its complementary strand were prepared as previously described [Naser et al. (1988) *Biochemistry* 27:4357-4367]. The uracil-containing oligonucleotide (U-37mer), the undamaged oligonucleotides and the complementary strand oligonucleotides for all the substrates were synthesized by the Emory University Microchemical Facility. The DHU-containing oligonucleotide (DHU-37mer) was synthesized by Research Genetics (Birmingham, AL). The oligonucleotides containing inosine (I-31mer) and xanthine (Xn-31mer) and their complementary strands were a gift from Dr. Yoke Wah Kow (Emory University, Atlanta, GA). The 8-oxoguanine-containing 37-mer (8-oxoG-37mer) was synthesized by National Biosciences Inc. (Plymouth, MN). Some oligonucleotide substrates were obtained from Operon, Inc., Alameda, CA.

Labeled oligonucleotide substrates were prepared as follows: The cs-CPD-30mer, the 49mer UV photodamage-containing oligonucleotides and the Pt-GG-32mer were 5' end-labeled with [γ-^{32}P] ATP (Amersham, 3000 Ci/mmol) using polynucleotide kinase [Tabor, S. (1985) In: *Current Protocols in Molecular Biology*, Greene Publishing Associates and Wiley (Interscience),

New York, NY). The oligonucleotides U-37mer, DHU-37mer, I-31mer, Xn-31mer and 8-oxoG-37mer were 3' end-labeled using terminal transferase (Promega, Madison, WI) and [α^{32}P] ddATP (Amersham, 3000 Ci/mmol) [Tu and Cohen (1980) *Gene* 10:177-183]. The end-labeled oligonucleotides were annealed to the appropriate complementary strand, and end-labeled duplex oligonucleotides were gel-purified on a 20% non-denaturing polyacrylamide gel. The DNA was resuspended in ddH_2O and stored at -20°C. The IDL or hairpin nature of the DNA substrates was also confirmed by monitoring DNA strand cleavage by purified *E. coli* endonuclease V, which cleaves the duplexes in a pattern specific for this enzyme [Yao and Kow (1994) *supra*; Yao and Kow (1996) *J. Biol. Chem.* 271:30672-30676].

The AP substrate was prepared as described hereinbelow. 5' end-labeled, duplex U-37mer (20-50 pmol) was incubated with uracil DNA glycosylase (UDG, 6 units) for 30 minutes at 37°C in UDG buffer (30 mM Hepes-KOH, pH 7.5, 1 mM EDTA, and 50 mM NaCl) to generate the AP site-containing oligonucleotide (AP-37mer). The DNA was extracted with PCIA (phenol-chloroform-isoamylalcohol, 29:19:1, v/v/v) equilibrated with HE buffer (10 mM Hepes-KOH pH 8.0, 2 mM EDTA) with 0.1% 8-hydroxyquinoline, and was evaluated for its AP site content by cleavage with 0.1 M piperidine at 90°C for 20 minutes.

The CPD-30mer Uve1p substrate [see herein and Kaur et al. (1998) *supra*] containing a centrally embedded, cis-syn TT cyclobutane pyrimidine dimer was a gift from John-Stephen Taylor (St. Louis, MO). All other oligonucleotide substrates (Table 1) for mismatch endonuclease experiments were synthesized by Operon, Inc. (Alameda, CA) or IDT, Inc. (Coralville, IA). All oligonucleotides were gel-purified and subjected to DNA sequence analysis for sequence confirmation. Oligonucleotides were 5' end-labeled with polynucleotide kinase using 50 μCi [γ-^{32}P] ATP (Amersham, 3000 Ci/mmol) as previously described [Bowman et al. (1994) *Nucl. Acids Res.* 22:3026-3032]. 3' end-labeled oligonucleotides were prepared by incubating 10 pmol of the indicated oligonucleotide with 10 units of terminal deoxynucleotidyl transferase (TdT, Promega) and 50 μCI of [α-^{32}P] ddATP (Amersham, 3000 Ci/mmol) as previously described [Bowman et al. (1994) *supra*].

Example 8. Establishment of optimal reaction conditions.

The optimal reaction conditions for UVDE cleavage of CPD-30mer were established by varying the NaCl concentration, divalent cation ($MnCl_2$, and $MgCl_2$) concentration, or by varying the pH of the reaction buffer in the reaction. The buffers (20 mM at the indicated pH range) were as follows: sodium citrate (pH 3-6), Hepes-KOH (pH 6.5-8), and sodium carbonate (pH 9-10.6). The optimum temperature required for enzyme activity was determined by pre-incubating the enzyme and the substrate in the reaction buffer at a specific temperature for 10 min prior to mixing UVDE and CPD-30mer. The reaction was stopped by phenol-chloroform-isoamyl alcohol extraction and the reaction products were analyzed on DNA sequencing gels as described above. From these experiments the following standard reaction conditions were established: 20 mM Hepes (pH 7.5), 100 mM NaCl, 10 mM $MgCl_2$, 1 mM $MnCl_2$, 30°C or at 37°C for 20 minutes.

Example 9. Kinetic assays

Enzyme reactions were carried out with 5 nM Δ228-UVDE or 11.5 nM GST-Δ228-UVDE in 20 mM Hepes (pH 6.5) in 10 mM $MgCl_2$, 1 mM $MnCl_2$, 100 mM NaCl. 5' End labeled CPD-30mer concentrations were varied from 25-250 nM in a final reaction volume of 15 μL for 0-3 minutes at 37° C. Initial enzyme velocities (V_i) were measured for each substrate concentration as nM of product formed per second. The apparent K_m, V_{max}, and turnover number (K_{cat}) were determined from Lineweaver Burk plots of averaged data (± standard deviations) from three independent experiments.

Example 10. Analysis of Uve1p Mismatch Repair Activity.

Reactions with GΔ228-Uve1p were carried out by incubating approximately 100 or 200 fmol of labeled oligonucleotide substrate with 100-150 ng of purified GΔ228-Uve1p in 20 mM Hepes (pH 6.5), 10 mM $MgCl_2$, 1 mM $MnCl_2$, and 150 mM NaCl for 15 or 20 minutes at 37°C (10-20 µl final volume). Reactions with crude preparations of GFL-Uve1p were carried out with 20-30 µg of cell extract incubated with the appropriate substrate in 20 mM Hepes (pH 7.5), 100 mM NaCl, 10 mM $MgCl_2$ and 1 mM $MnCl_2$ at 37°C for 20 minutes. The reaction products were

processed by extracting with an equal volume of phenol-chloroform-isoamyl alcohol (25:24:1), ethanol-precipitation, resuspension and analysis on 20% denaturing (7 M urea) polyacrylamide (DNA sequencing) gels as previously described [Kaur et al. (1998) *supra*]. The DNA species corresponding to the uncleaved substrate and Uve1p-mediated DNA strand scission products were analyzed and quantified by phosphorimager analysis (Molecular Dynamics model 445SI) and/or autoradiography. Base specific chemical cleavage DNA sequencing reaction products were included in the gels as size markers.

Terminal analysis of the mismatch cleavage products was carried out as follows. GΔ228-Uve1p was incubated with 3' end-labeled *CX/AY-31mer under standard reaction conditions at 37°C for 20 minutes. The ethanol-precipitated reaction products were incubated with either 10 units of calf intestinal phosphatase (CIP, Promega, Madison WI) at 37°C for 30 minutes or with 10 units of T4 polynucleotide kinase (PNK, New England Biolabs) and 50 pmol ATP as previously described [Bowman et al. (1994) *supra*]. The reaction products were analyzed on 20% denaturing polyacrylamide gels as described above for Uve1p activity assays. Differences in electrophoretic mobilities of kinase-treated versus untreated DNA strand scission products indicated the presence or absence of a pre-existing 5'-phosphoryl group [Bowman et al. (1994) *supra*].

3' terminal analysis of the mismatch cleavage products was carried out as follows. To determine the chemical nature of the 3' terminus of GSTΔ228-Uve1p-mediated DNA strand scission products, 5' end-labeled *CX/AY-31mer was incubated with GΔ228-Uve1p as described above. The ethanol-precipitated, resuspended reaction products were then treated with 10 units of TdT and ddATP as previously described [Bowman et al (1994) *supra*]. Samples were processed and analyzed on polyacrylamide gels as described above for 5' terminal analysis.

To determine the pH optimum for Uve1p-mediated mismatch cleavage, 100 fmol of 3' end-labeled *CX/AY-31mer was incubated with approximately 100 ng of GΔ228-Uve1p with 10 mM $MgCl_2$ and 1 mM $MnCl_2$ in 20 mM reaction buffer of different pH ranges (pH 3.0-10.6).

The buffers were as follows: sodium citrate (pH 3.0-6.0), Hepes-KOH (pH 6.5-8.0), and sodium carbonate (pH 9.0-10.6). The reaction products were analyzed on a 20% denaturing polyacrylamide gel and the optimal pH was calculated as previously described for Uve1p cleavage of CPD-30mer [Kaur et al. (1998) *supra*].

For substrate competition assays, end-labeled *CX/AY-31mer was generated by annealing 3' end-labeled CX with unlabeled strand AY. Unlabeled non-specific (non-mismatch) competitor GX/CY-31mer was made by annealing strand GX to strand CY resulting in a duplex oligonucleotide with a C/A base pair instead of a G/G mispair. CPD-30mer, a well-characterized substrate for Uve1p, was employed as an unlabeled, specific competitor. 3' end-labeled *CX/AY-31mer (0.1 pmol) was incubated with 100 ng of purified GΔ228-Uve1p and increasing amounts (0.1-2.0 pmol) of either specific (CPD-30mer) or non-specific (GX/CY-31mer) competitor. The competition reactions were processed and analyzed on 20% denaturing gels as described above. The DNA species corresponding to the uncleaved *GX/GY-31mer and the DNA strand scission products were quantified by phosphorimager analysis (Molecular Dynamics model 445SI).

Example 11. Mutation Frequencies Assayed by Canavanine Resistance.

To determine sensitivity to L-canavanine, 10 ml of PMALU^g was inoculated with 100 µl of the indicated saturated culture and grown to mid-log phase at 25°C. 200 cells were plated onto PMALU^g plates with varying concentrations of L-canavanine sulfate (0, 0.075, 0.22, 0.75, 2.2, 7.5, 22, and 75 µg/ml) and incubated at 30°C. Colonies were counted after four days and viability was normalized against the 0 g/ml plate for each strain. Colony formation assays were conducted for each strain by plating 10^7 cells from saturated cultures onto PMALU^g plates supplemented with 75 µg/ml L-canavanine sulfate. Colonies were counted after eight days incubation at 30°C. Mean mutation frequencies were calculated using the method of the median as described by Lea and Coulson (1943) *J. Genet.* 49:264-284.

Example 12. Antibody Inhibition.

The N-terminal glutathione-S-transferase tag was removed from GΔ228-Uve1p by thrombin cleavage, and Δ228-Uve1p was purified as described previously [Kaur et al. (1998) *supra*]. Rabbit polyclonal antibodies were raised using purified Δ228-Uve1p as immunogen. GΔ228-Uve1p was incubated with immune or pre-immune sera for 45 min. at 4°C and then incubated with the indicated end-labeled duplex oligonucleotides for Uve1p assay as described above. The reaction products were analyzed as described above.

Table 1A. Damaged Oligonucleotide Substrates Used in This Study.

cis-syn cyclobutane pyrimidine dimers (cs-CPDs), *trans-syn* I CPD (tsI-CPD), *trans-syn* II CPD (tsII-CPD), (6-4) photoproducts (6-4PP), a Dewar isomer (Dewar), a platinum DNA diadduct (Pt-GG), uracil (U), dihydrouracil (DHU), abasic site (AP), inosine (I), xanthine (Xn) and 8-oxoguanine (8-oxoG).

Substrate	Damaged oligonucleotide sequence 5' to 3'	Adduct	[a]Opposite base(s).	SEQ ID NO.:
A: cs-CPD-30mer	CATGCCTGCACGAAT^TAAGCAATTCGTAAT	cs-CPD	AA	13
B: UD-30mer	CATGCCTGCACGAATTAAGCAATTCGTAAT	undamaged	AA	14
C: cs-CPD-49mer	AGCTACCATGCCTGCACGAAT^TAAGCAATTCGTAATCATGGTCATAGCT	cs-CPD	AA	15
D: tsI-CPD-49mer	"	tsI-CPD	AA	16
E: tsII-CPD-49mer	"	tsII-CPD	AA	17
F: 6-4PP-49mer	"	6-4PP	AA	18
G: Dewar-49mer	"	Dewar	AA	19
H: Pt-GG-32mer	TCCCTCCTTCCTTCC**G*G***CCCTCCTTCCCCTTC	Pt-GG	CC	20
I: U-37mer	CTTGGACTGGATGTCGGCAC**X**AGCGGATACAGGAGCA	U	A/G	21
J: DHU-37mer	"	DHU	A/G	22
K: AP-37mer	"	AP	A/G	23
L: I-31mer	TGCAGGTCGACT**X**AGGAGGATCCCCGGGTAC	I	T/C	24
M: Xn-31mer	"	Xn	T/C	25
N: 8-oxoG-37mer	CTTGGACTGGATGTCGGCAC**X**AGCGGATACAGGAGCA	8-oxoG	A/T/G/C	26

[a]denotes the bases that are placed opposite to the lesions on the complementary DNA strand.

^, *, X represent a UV induced dimer between two adjacent thymines, a cisplatin induced diadduct between two adjacent guanines and position at which the adducts U, DHU, AP, I, Xn and 8-oxoG are incorporated into the oligonucleotide substrates, respectively.

Table 1B: Base Mismatch and CPD-Containing Oligonucleotides Used in This Study.

Oligo Name	Sequence	Strand Designation	SEQ ID NO:
XY-31mer[1]	5' GTACCCGGGGATCCTCC**X**AGTCGACCTGCA 3' 3' CATGGGCCCCTAGGAGG**Y**TCAGCTGGACGT 5'	GX, AX, TX, CX: X=G, A, T, C GY, AY, TY, CY: Y=G, A, T, C	27
CX/AY-41mer	5' CGTTAGCATGCCTGCACGAA**C**TAAGCAATTCGTAATGCATT 3' 3' GCAATCGTACGGACGTGCTT**A**ATTCGTTAAGCATTACGTAA 5'	CX AY	28
C(6)/A-41mer[2]	5' CGTTACAAGTCCGTCACGAATTAAGCAATTCGTAA**C**GCATT 3' 3' GCAATGTTCAGGCAGTGCTTAATTCGTTAAGCATT**A**CGTAA 5'	C(6) A(36)	29
C(11)/A-41mer	5' CGTTACAAGTCCGTCACGAATTAAGCAATT**C**GTAACGCATT 3' 3' GCAATGTTCAGGCAGTGCTTAATTCGTTAAA**C**ATTGCGTAA 5'	C(11) A(31)	30
C(16)/A-41mer	5' CGTTACAAGTCCGTCACGAATTAAG**C**AATTCGTAACGCATT 3' 3' GCAATGTTCAGGCAGTGCTTAATTC**A**TTAAGCATTGCGTAA 5'	C(16) A(26)	31
C(22)/A-41mer	5' CGTTACAAGTCCGTCACGA**C**TTAAGCAATTCGTAACGCATT 3' 3' GCAATGTTCAGGCAGTGCT**A**AATTCGTTAAGCATTGCGTAA 5'	C(22) A(20)	32
C(27)/A-41mer	5' CGTTACAAGTCCGT**C**ACGAATTAAGCAATTCGTAACGCATT 3' 3' GCAATGTTCAGGCAA**T**GCTTAATTCGTTAAGCATTGCGTAA 5'	C(27) A(15)	33
C(32)/A-41mer	5' CGTTACAAG**C**CCGTCACGAATTAAGCAATTCGTAACGCATT 3' 3' GCAATGTTC**A**GGCAGTGCTTAATTCGTTAAGCATTGCGTAA 5'	C(32) A(10)	34
C(37)/A-41mer	5' CGTT**C**CAAGTCCGTCACGAATTAAGCAATTCGTAACGCATT 3' 3' GCAA**A**GTTCAGGCAGTGCTTAATTCGTTAAGCATTGCGTAA 5'	C(37) A(5)	35
CPD-30mer[3]	5' CATGCCTGCACGAA**T^T**AAGCAATTCGTAAT 3' 3' GTACGGACGTGCTTA ATTCGTTAAGCATTA 5'	30 D 30 C	13

[1]Series of 16 different duplex oligos containing all possible base pair/mispair combinations between G, A, T, and C.
In text, * denotes labeled strand (e.g. *CX/AY-31mer corresponds to C/A mismatch with the C-containing X strand as the labeled strand).
[2]C/A mismatch oligos designated by base position of the mismatched C from the 3' terminus.
[3]CPD-30mer contains a cyclobutane pyrimidine dimer designated as **T^T**.

Table 2. Activity of Uve1p on Oligonucleotide Substrates Containing Uracil, Dihydrouracil and AP sites

Protein	U/G	U/A	DHU/G	DHU/A	AP/G	AP/A
[a]Positive control	90-100	50-60	70-80	15-20	90-100	90-100
GΔ228-Uve1p	8-12	1-5	37-42	10-15	90-100	90-100
GST	1-5	1-5	1-5	1-5	1-5	1-5

The percent of substrate converted into total DNA cleavage products formed when the DNA damage lesion is base paired with a G or an A in the complementary strand. Details of experiments are outlined in Example 10.

[a]Positive control: when analyzing U 37mer, uracil DNA glycosylase (UDG) was used as a positive control; for assays involving DHU 37mer, the *S. cerevisiae* endonuclease III-like homolog Ntg1 was used as a positive control; *E. coli* endonuclease IV was used as a positive control for AP endonuclease activity.

Table 3. Uve1p Cleavage Efficiency on Different Substrates.

Substrate	Percent [Cleavage]
cs-CPD 49mer	89
tsI-CPD 49mer	75
tsII-CPD 49mer	75
6-4PP 49mer	71
Dewar	83
AP 37mer	12.5
DHU 37mer	3
Pt-GG 32mer	2.5
U 37mer	1
8-oxoG 37mer	0
I 31mer	0
Xn 31mer	0

[a]The percent cleavage was calculated by quantifying the amount of Uve1p-mediated cleavage product formed when 300 ng of affinity-purified GΔ228-Uve1p was incubated with ~150 fmol of each substrate.

Table 4. Spontaneous Mutation Rates of *uve1* and *pms1* Null Mutants

Genotype	Distribution of canavanine-resistant colonies/plate				Median no. of colonies/10^7 cells	Calculated mutation frequency (mean ± SE)
	0-2	3-34	35-86	>86		
Wild type	18	16	2	0	2.5	$1.5 \times 10^{-7} \pm 2.5 \times 10^{-8}$
uve1::ura4$^+$	4	14	8	10	34.5	$9.7 \times 10^{-7} \pm 4.2 \times 10^{-8}$
pms1::ura4$^+$	0	8	10	18	86.5	$2.0 \times 10^{-6} \pm 5.0 \times 10^{-8}$

Table 5. Nucleotide Sequence Encoding GST-Full-length UVDE (SEQ ID NO:1)

```
   1  atgaccaagt tacctatact aggttattgg aaaaattaag ggccttgtgc
  51  aacccactcg acttcttttg gaatatcttg aagaaaaata tgaagagcat
 101  ttgtatgagc gcgatgaagg tgataaatgg cgaaacaaaa agtttgaatt
 151  gggtttggag tttcccaatc ttccttatta tattgatggt gatgttaaat
 201  taacacagtc tatggccatc atacgttata tagctgacaa gcacaacatg
 251  ttggttggtt gtccaaaaga gcgtgcagag atttcaatgc ttgaaggagc
 301  ggttttggat attagatacg gtgtttcgag aattgcatat agtaaagact
 351  ttgaaactct caaagttgat tttcttagca agctacctga aatgctgaaa
 401  atgttcgaag atcgtttatg tcataaaaca tatttaaatg ttgaccatgt
 451  aacccatcct gacttcatgt tgtatgacgc tcttgatgtt gttttataca
 501  tggacccaat gtgcctggat gcgttcccaa aattagtttg ttttaaaaaa
 551  cgtattgaag ctatcccaca aattgataag tacttgaaat ccagcaagta
 601  tatagcatgg cctttgcagg gctggcaagc cacgtttggt ggtggcgacc
 651  atcctccaaa atcggatcat ctggttccgc gtggatccat gcttaggcta
 701  ttgaaacgaa atattcaaat ctctaaacgc attgttttca ccatattaaa
 751  acaaaaggca tttaaaggta atcatccttg tgtaccgtcg gtttgtacca
 801  ttacttactc tcgttttcat tgtttacccg ataccccttaa aagtttactt
 851  ccaatgagct caaaaaccac actctcaatg ttaccgcaag ttaatatcgg
 901  tgcgaattca ttctctgccg aaacaccagt cgacttaaaa aaagaaaatg
 951  agactgagtt agctaatatc agtggacctc acaaaaaaag tacttctacg
1001  tctacacgaa agagggcacg tagcagtaaa aagaaagcga cagattctgt
1051  ttccgataaa attgatgagt ctgttgcgtc ctatgattct tcaactcatc
1101  ttaggcgatc gtcgagatca aaaaaaccgg tcaactacaa ttcctcgtca
```

Table 5. Continued

```
1151  gaatccgaat cggaggagca aattagtaaa gctactaaaa aagttaaaca
1201  aaaagaggaa gaggagtatg ttgaagaagt cgacgaaaag tctcttaaaa
1251  atgaaagtag ctctgacgag ttcgaaccgg ttgtgccgga acagttggaa
1301  actccaattt ctaaacgaag acggtctcgt tcttctgcaa aaaatttaga
1351  aaaagaatct acaatgaatc ttgatgatca tgctccacga gagatgtttg
1401  attgtttgga caaacccata ccctggcgag gacgattggg gtatgcttgt
1451  ttgaatacta ttttaaggtc aatgaaggag agggtttttt gttcacgcac
1501  ctgccgaatt acaaccattc aacgtgatgg gctcgaaagt gtcaagcagc
1551  taggtacgca aaatgtttta gatttaatca aattggttga gtggaatcac
1601  aactttggca ttcacttcat gagagtgagt tctgatttat ttcctttcgc
1651  aagccatgca aagtatggat ataccttga atttgcacaa tctcatctcg
1701  aggaggtggg caagctggca aataaatata atcatcgatt gactatgcat
1751  cctggtcagt acacccagat agcctctcca cgagaagtcg tagttgattc
1801  ggcaatacgt gatttggctt atcatgatga aattctcagt cgtatgaagt
1851  tgaatgaaca attaaataaa gacgctgttt taattattca ccttggtggt
1901  acctttgaag gaaaaaaaga aacattggat aggtttcgta aaaattatca
1951  acgcttgtct gattcggtta aagctcgttt agttttagaa aacgatgatg
2001  tttcttggtc agttcaagat ttattacctt tatgccaaga acttaatatt
2051  cctctagttt tggattggca tcatcacaac atagtgccag gaacgcttcg
2101  tgaaggaagt ttagatttaa tgccattaat cccaactatt cgagaaacct
2151  ggacaagaaa gggaattaca cagaagcaac attactcaga atcggctgat
2201  ccaacggcga tttctgggat gaaacgacgt gctcactctg atagggtgtt
2251  tgactttcca ccgtgtgatc ctacaatgga tctaatgata gaagctaagg
2301  aaaaggaaca ggctgtattt gaattgtgta gacgttatga gttacaaaat
2351  ccaccatgtc ctcttgaaat tatggggcct gaatacgatc aaactcgaga
2401  tggatattat ccgcccggag ctgaaaagcg tttaactgca agaaaaaggc
2451  gtagtagaaa agaagaagta gaagaggatg aaaaataaaa at
```

Table 6. Deduced Amino Acid Sequences of GST-Full-length UVDE (SEQ ID NO:2)

```
  1  mtklpilgyw kikglvqptr llleyleeky eehlyerdeg dkwrnkkfel
 51  glefpnlpyy idgdvkltqs maiiryiadk hnmlggcpke raeismlega
101  vldirygvsr iayskdfetl kvdflsklpe mlkmfedrlc hktylngdhv
151  thpdfmlyda ldvvlymdpm cldafpklvc fkkrieaipq idkylkssky
201  iawplqgwqa tfgggdhppk sdhlvprgsm lrllkrniqi skrivftilk
251  qkafkgnhpc vpsvctitys rfhclpdtlk sllpmssktt lsmlpqvnig
301  ansfsaetpv dlkkenetel anisgphkks tststrkrar sskkkatdsv
351  sdkidesvas ydssthlrrs srskkpvnyn sssese seeq iskatkkvkq
401  keeeeyveev dekslkness sdefepvvpe qletpiskrr rsrssaknle
451  kestmnlddh apremfdcld kpipwrgrlg yaclntilrs mkervfcsrt
501  crittiqrdg lesvkqlgtq nvldliklve wnhnfgihfm rvssdlfpfa
551  shakygytle faqshleevg klankynhrl tmhpgqytqi aspievvvds
601  airdlayhde ilsrmklneq lnkdavliih lggtfegkke tldrfrknyq
651  rlsdsvkarl vlenddvsws vqdllplcqe lniplvldwh hhnivpgtlr
701  egsldlmpli ptiretwtrk gitqkqhyse sadptaisgm krrahsdrvf
751  dfppcdptmd lmieakekeq avfelcrrye lqnppcplei mgpeydqtrd
801  gyyppgaekr ltarkrrsrk eeveedek
```

Table 7. Nucleotide Sequence Encoding Δ228-UVDE (SEQ ID NO:3)

```
   1  gatgatcatg ctccacgaga gatgtttgat tgtttggaca aacccatacc
  51  ctggcgagga cgattggggt atgcttgttt gaatactatt ttaaggtcaa
 101  tgaaggagag ggtttttttgt tcacgcacct gccgaattac aaccattcaa
 151  cgtgatgggc tcgaaagtgt caagcagcta ggtacgcaaa atgttttaga
 201  tttaatcaaa ttggttgagt ggaatcacaa ctttggcatt cacttcatga
 251  gagtgagttc tgatttattt cctttcgcaa gccatgcaaa gtatggatat
 301  acccttgaat ttgcacaatc tcatctcgag gaggtgggca agctggcaaa
 351  taaatataat catcgattga ctatgcatcc tggtcagtac acccagatag
 401  cctctccacg agaagtcgta gttgattcgg caatacgtga tttggcttat
 451  catgatgaaa ttctcagtcg tatgaagttg aatgaacaat taaataaaga
 501  cgctgtttta attattcacc ttggtggtac ctttgaagga aaaaaagaaa
 551  cattggatag gtttcgtaaa aattatcaac gcttgtctga ttcggttaaa
 601  gctcgtttag ttttagaaaa cgatgatgtt tcttggtcag ttcaagattt
 651  attaccttta tgccaagaac ttaatattcc tctagttttg gattggcatc
 701  atcacaacat agtgccagga acgcttcgtg aaggaagttt agatttaatg
 751  ccattaatcc caactattcg agaaacctgg acaagaaagg gaattacaca
 801  gaagcaacat tactcagaat cggctgatcc aacggcgatt tctgggatga
 851  aacgacgtgc tcactctgat agggtgtttg actttccacc gtgtgatcct
 901  acaatggatc taatgataga agctaaggaa aaggaacagg ctgtatttga
 951  attgtgtaga cgttatgagt tacaaaatcc accatgtcct cttgaaatta
1001  tggggcctga atacgatcaa actcgagatg gatattatcc gcccggagct
1051  gaaaagcgtt taactgcaag aaaaaggcgt agtagaaaag aagaagtaga
1101  agaggatgaa aaataaaaat ccgtcatact ttttgattta tggcataatt
1151  tagccatctc c
```

Table 8. Deduced Amino Acid Sequence of Δ228-UVDE (SEQ ID NO:4)

```
  1  ddhapremfd cldkpipwrg rlgyaclnti lrsmkervfc srtcrittiq
 51  rdglesvkql gtqnvldlik lvewnhnfgi hfmrvssdlf pfashakygy
101  tlefaqshle evgklankyn hrltmhpgqy tqiasprevv vdsairdlay
151  hdeilsrmkl neqlnkdavl iihlggtfeg kketldrfrk nyqrlsdsvk
201  arlvlenddv swsvqdllpl cqelniplvl dwhhhnivpg tlregsldlm
251  pliptiretw trkgitqkqh ysesadptai sgmkrrahsd rvfdfppcdp
301  tmdlmieake keqavfelcr ryelqnppcp leimgpeydq trdgyyppga
351  ekrltarkrr srkeeveede k
```

Table 9. Nucleotide Sequence Encoding GST-Δ228-UVDE (SEQ ID NO:5)

```
   1  atgaccaagt tacctatact aggttattgg aaaaattaag ggccttgtgc
  51  aacccactcg acttcttttg gaatatcttg aagaaaaata tgaagagcat
 101  ttgtatgagc gcgatgaagg tgataaatgg cgaaacaaaa agtttgaatt
 151  gggtttggag tttcccaatc ttccttatta tattgatggt gatgttaaat
 201  taacacagtc tatggccatc atacgttata tagctgacaa gcacaacatg
 251  ttggttggtt gtccaaaaga gcgtgcagag atttcaatgc ttgaaggagc
 301  ggttttggat attagatacg gtgtttcgag aattgcatat agtaaagact
 351  ttgaaactct caaagttgat tttcttagca agctacctga aatgctgaaa
 401  atgttcgaag atcgtttatg tcataaaaca tatttaaatg ttgaccatgt
 451  aacccatcct gacttcatgt tgtatgacgc tcttgatgtt gttttataca
 501  tggacccaat gtgcctggat gcgttcccaa aattagtttg ttttaaaaaa
 551  cgtattgaag ctatcccaca aattgataag tacttgaaat ccagcaagta
 601  tatagcatgg cctttgcagg gctggcaagc cacgtttggt ggtggcgacc
 651  atcctccaaa atcggatcat ctggttccgc gtggatccga tgatcatgct
 701  ccacgagaga tgtttgattg tttggacaaa cccatаccct ggcgaggacg
 751  attggggtat gcttgtttga atactatttt aaggtcaatg aaggagaggg
 801  ttttttgttc acgcacctgc cgaattacaa ccattcaacg tgatgggctc
 851  gaaagtgtca agcagctagg tacgcaaaat gttttagatt taatcaaatt
 901  ggttgagtgg aatcacaact ttggcattca cttcatgaga gtgagttctg
 951  atttatttcc tttcgcaagc catgcaaagt atggatatac ccttgaattt
1001  gcacaatctc atctcgagga ggtgggcaag ctggcaaata aatataatca
1051  tcgattgact atgcatcctg gtcagtacac ccagatagcc tctccacgag
1101  aagtcgtagt tgattcggca atacgtgatt tggcttatca tgatgaaatt
```

Table 9. Continued

```
1151  ctcagtcgta tgaagttgaa tgaacaatta aataaagacg ctgttttaat
1201  tattcacctt ggtggtacct ttgaaggaaa aaaagaaaca ttggataggt
1251  ttcgtaaaaa ttatcaacgc ttgtctgatt cggttaaagc tcgtttagtt
1301  ttagaaaacg atgatgtttc ttggtcagtt caagatttat tacctttatg
1351  ccaagaactt aatattcctc tagttttgga ttggcatcat cacaacatag
1401  tgccaggaac gcttcgtgaa ggaagtttag atttaatgcc attaatccca
1451  actattcgag aaacctggac aagaaaggga attacacaga agcaacatta
1501  ctcagaatcg gctgatccaa cggcgatttc tgggatgaaa cgacgtgctc
1551  actctgatag ggtgtttgac tttccaccgt gtgatcctac aatggatcta
1601  atgatagaag ctaaggaaaa ggaacaggct gtatttgaat tgtgtagacg
1651  ttatgagtta caaaatccac catgtcctct tgaaattatg gggcctgaat
1701  acgatcaaac tcgagatgga tattatccgc ccggagctga aaagcgttta
1751  actgcaagaa aaaggcgtag tagaaaagaa gaagtagaag aggatgaaaa
1801  ataaggatcc c
```

Table 10. Deduced Amino Acid Sequence of GST-Δ228-UVDE (SEQ ID NO:6)

```
  1  mtklpilgyw kikglvqptr llleyleeky eehlyerdeg dkwrnkkfel
 51  glefpnlpyy idgdvkltqs maiiryiadk hnmlggcpke raeismlega
101  vldirygvsr iayskdfetl kvdflsklpe mlkmfedrlc hktylngdhv
151  thpdfmlyda ldvvlymdpm cldafpklvc fkkrieaipq idkylkssky
201  iawplqgwqa tfgggdhppk sdhlvprgsd dhapremfdc ldkpipwrgr
251  lgyaclntil rsmkervfcs rtcrittiqr dglesvkqlg tqnvldlikl
301  vewnhnfgih fmrvssdlfp fashakygyt lefaqshlee vgklankynh
351  rltmhpgqyt qiasprevvv dsairdlayh deilsrmkln eqlnkdavli
401  ihlggtfegk ketldrfrkn yqrlsdsvka rlvlenddvs wsvqdllplc
451  qelniplvld whhhnivpgt lregsldlmp liptiretwt rkgitqkqhy
501  sesadptais gmkrrahsdr vfdfppcdpt mdlmieakek eqavfelcrr
551  yelqnppcpl eimgpeydqt rdgyyppgae krltarkrrs rkeeveedek
```

Table 11. Nucleotide Sequence Encoding the GST Leader Sequence (SEQ ID NO:7)

```
  1  atgaccaagt tacctatact aggttattgg aaaaattaag ggccttgtgc
 51  aacccactcg acttcttttg gaatatcttg aagaaaaata tgaagagcat
101  ttgtatgagc gcgatgaagg tgataaatgg cgaaacaaaa agtttgaatt
151  gggtttggag tttcccaatc ttccttatta tattgatggt gatgttaaat
201  taacacagtc tatggccatc atacgttata tagctgacaa gcacaacatg
251  ttggttggtt gtccaaaaga gcgtgcagag atttcaatgc ttgaaggagc
301  ggttttggat attagatacg gtgtttcgag aattgcatat agtaaagact
351  ttgaaactct caaagttgat tttcttagca agctacctga aatgctgaaa
401  atgttcgaag atcgtttatg tcataaaaca tatttaaatg ttgaccatgt
451  aacccatcct gacttcatgt tgtatgacgc tcttgatgtt gttttataca
501  tggacccaat gtgcctggat gcgttcccaa aattagtttg ttttaaaaaa
551  cgtattgaag ctatcccaca aattgataag tacttgaaat ccagcaagta
601  tatagcatgg cctttgcagg gctggcaagc cacgtttggt ggtggcgacc
651  atcctccaaa atcggatcat ctggttccgc gtggatcc
```

Table 12. Deduced Amino Acid Sequence of the GST Leader Polypeptide (SEQ ID NO:8)

```
  1  MTKLPILGYW KIKGLVQPTR LLLEYLEEKY EEHLYERDEG DKWRNKKFEL
 51  GLEFPNLPYY IDGDVKLTQS MAIIRYIADK HNMLGGCPKE RAEISMLEGA
101  VLDIRYGVSR IAYSKDFETL KVDFLSKLPE MLKMFEDRLC HKTYLNGDHV
151  THPDFMLYDA LDVVLYMDPM CLDAFPKLVC FKKRIEAIPQ IDKYLKSSKY
201  IAWPLQGWQA TFGGGDHPPK SDHLVPRGS
```

Table 13. Neospora crassa UVDE Homolog (Genbank Accession No. BAA 74539) (SEQ ID NO:36)

mpsrkskaaaldtpqsesstfsstldssapsparnlrrsgrnilqpssekdrdhekrsgeelagrmmgkda
nghclregkeqeegvkmaieglarmerrlqratkrqkkqleedgipvpsvvsrfptapyhhkstnaeere
akepvlkthskdvereaeigvddvvkmepaatniiepedaqdaaergaarppavnssylplpwkgrlg
yaclntylrnskppifssrtcrmasivdhrhplqfedepehhlknkpdkskepqdelghkfvqelglanar
divkmlcwnekygirflrlssemfpfashpvhgyklapfasevlaeagrvaaelghrltthpgqftqlgsp
rkevvesairdleyhdellsllklpeqqnrdavmiihmggqfgdkaatlerfkrnyarlsqscknrlvlend
dvgwtvhdllpvceelnipmvldyhhhnicfdpahlregtldisdpklqeriantwkrkgikqkmhyse
pcdgavtprhrrkhrprvmtlppcppdmdlmieakdkeqavfelmrtfklpgfekindmvpydrdde
nrpappvkapkkkkggkrkrttdeeaaepeevdtaaddvkdapegpkevpeeeramggpynrvyw
plgceewlkpkkrevkkgkvpeevedegefdg

Table 14. Bacillus subtilis UVDE Homolog (Genbank Accession No. Z 49782) (SEQ ID NO:37)

mifrfgfvsnamslwdaspaktltfarysklskterkealltvtkanlrntmrtlhyiighgiplyrfsssivpl
athpdvmwdfvtpfqkefreigelvkthqlrtsfhpnqftlftspkesvtknavtdmayhyrmleamgia
drsvinihiggaygnkdtataqfhqnikqlpqeikermtlenddktytteetlqvceqedvpfvfdfhhfy
anpddhadlnvalprmiktweriglqpkvhlsspkseqairshadyvdanfllpllerfrqwgtnidfmie
akqkdkallrlmdelssirgvkrigggalqwks

Table 15. Human UVDE Homolog (Genbank Accession No. AF 114784.1) (SEQ ID NO:38)

```
  1 mgttglesls lgdrgaaptv tsserlvpdp pndlrkedva melervgede eqmmikrsse
 61 cnpllqepia saqfgatagt ecrksvpcgw ervvkqrlfg ktagrfdvyf ispqglkfrs
121 ksslanylhk ngetslkped fdftvlskrg iksrykdcsm aaltshlqnq snnsnwnlrt
181 rskckkdvfm ppsssselqe srglsnftst hlllkedegv ddvnfrkvrk pkgkvtilkg
241 ipikktkkgc rkscsgfvqs dskresvcnk adaesepvaq ksqldrtvci sdagacgetl
301 svtseenslv kkkerslssg snfcseqkts giinkfcsak dsehnekyed tfleseeigt
361 kvevverkeh lhtdilkrgs emdnncsptr kdftgekifq edtiprtqie rrktslyfss
421 kynkealspp rrkafkkwtp prspfnlvqe tlfhdpwkll iatiflnrts gkmaipvlwk
481 flekypsaev artadwrdvs ellkplglyd lraktivkfs deyltkqwky pielhgigky
541 gndsyrifcv newkqvhped hklnkyhdwl wenheklsls
```

Table 16. D. radiodurans UVDE Homolog (SEQ ID NO:39)

```
  1  QLGLVCLTVG PEVRFRTVTL SRYRALSPAE REAKLLDLYS SNIKTLRGAA

 51  DYCAAHDIRL YRLSSSLFPM LDLAGDDTGA AVLTHLAPQL LEAGHAFTDA

101  GVRLLMHPEQ FIVLNSDRPE VRESSVRAMS AHARVMDGLG LARTPWNLLL

151  LHGGKGGRGA ELAALIPDLP DPVRLRLGLE NDERAYSPAE LLPICEATGT

201  PLVFDAHHHV VHDKLPDQED PSVREWVLRA RATWQPPEWQ VVHLSNGIEG

251  PQDRRHSHLI ADFPSAYADV PWIEVEAKGK EEAIAALRLM APFK
```

Tables 17A-17B: Potential IDL substrates for Uve1p. Sequences of the oligonucleotide strands utilized to generate the heteroduplex substrates used in this study. Names of individual oligonucleotide strands are indicated beside each duplex. Two different sequence types (I and Lo) were used.

Table 17A. Duplex type ID substrates containing IDL loops of 0, 2, 4, 6, 8 and 16 nt in length.

		Strand	SEQ ID NO
5'	CACAGACTCCCTCTGTCATAGGTTTGAGTTTATATGGAA 3'	IX	40
3'	GTGTCTGAGGGAGACAGTATCCAAACTCAAATATACCTT 5'	D0	41
5'	CACAGACTCCCTCTGTCATAGGTTCATGAGTTTATATGGAA 3'	ID2	42
3'	GTGTCTGAGGGAGACAGTATCCAA--ACTCAAATATACCTT 5'	D0	41
5'	CACAGACTCCCTCTGTCATAGGTTCACATGAGTTTATATGGAA 3'	ID4	43
3'	GTGTCTGAGGGAGACAGTATCCAA----ACTCAAATATACCTT 5'	D0	41
5'	CACAGACTCCCTCTGTCATAGGTTCACACATGAGTTTATATGGAA 3'	ID6	44
3'	GTGTCTGAGGGAGACAGTATCCAA------ACTCAAATATACCTT 5'	D0	41
5'	CACAGACTCCCTCTGTCATAGGTTCACACACATGAGTTTATATGGAA 3'	ID8	45
3'	GTGTCTGAGGGAGACAGTATCCAA--------ACTCAAATATACCTT 5'	D0	41
5'	CACAGACTCCCTCTGTCATAGGTTGAGTACTAGTACTCTGAGTTTATATGGAA 3'	HP8	46
3'	GTGTCTGAGGGAGACAGTATCCAAACTCAAATATACCTT 5'	D0	41

Table 17B. Duplex type LD substrates containing IDL loops of 0, 2, 4, 6 and 8 nt in length.

		Strand	SEQ ID NO
5'-CGTTAGAACTCCGTCACGAATTAAGCAATTAGTAATGCATT 3'		Lo0	47
3'-GCAATCTTGAGGCAGTGCTTAATTCGTTAATCATTACGTAA 5'		Bot	48
5'-CGTTAGAACTCCGTCACGAATTAAGCAATTCAAGTAATGCATT 3'		Lo2	49
3'-GCAATCTTGAGGCAGTGCTTAATTCGTTAA--TCATTACGTAA 5'		Bot	48
5'-CGTTAGAACTCCGTCACGAATTAAGCAATTCACAAGTAATGCATT 3'		Lo4	50
3'-GCAATCTTGAGGCAGTGCTTAATTCGTTAA----TCATTACGTAA 5'		Bot	48
5'-CGTTAGAACTCCGTCACGAATTAAGCAATTCACACAAGTAATGCATT 3'		Lo6	51
3'-GCAATCTTGAGGCAGTGCTTAATTCGTTAA-----=TCATTACGTAA 5'		Bot	48
5'-CGTTAGAACTCCGTCACGAATTAAGCAATTCACACACAAGTAATGCATT 3'		Lo8	52
3'-GCAATCTTGAGGCAGTGCTTAATTCGTTAA--------TCATTACGTAA 5'		Bot	48

Table 18. Sequences of oligonucleotides used in strand specificity experiments.

1	5'-CGTTACAAGTCCGTCACGAATTAAGCAATTCGTAA**C**GCATT-3'	C6, SEQ ID NO:53
	3'-GCAATGTTCAGGCAGTGCTTAATTCGTTAAGCATT**A**CGTAA-5'	A36, SEQ ID NO:54
	5-AATGCATTACGAATTGCTTAATTCGTGACGGACTTGTAACG*	
2	5'-CGTTACAAGTCCGTCACGAATTAAGCAATT**C**GTAACGCATT-3'	C11, SEQ ID NO:55
	3'-GCAATGTTCAGGCAGTGCTTAATTCGTTAA**A**CATTGCGTAA-5'	A31, SEQ ID NO:56
	AATGCGTTACAAATTGCTTAATTCGTGACGGACTTGTAACG*	
3	5'-CGTTACAAGTCCGTCACGAATTAAG**C**AATTCGTAACGCATT-3'	C16, SEQ ID NO:57
	3'-GCAATGTTCAGGCAGTGCTTAATTC**A**TTAAGCATTGCGTAA-5'	A26, SEQ ID NO:58
	AATGCGTTACGAATTACTTAATTCGTGACGGACTTGTAACG*	
4	5'-CGTTACAAGTCCGTCACGA**C**TTAAGCAATTCGTAACGCATT-3'	C22, SEQ ID NO:59
	3'-GCAATGTTCAGGCAGTGCT**A**AATTCGTTAAGCATTGCGTAA-5'	A20, SEQ ID NO:60
	AATGCGTTACGAATTGCTTAAATCGTGACGGACTTGTAACG*	
5	5'-CGTTACAAGTCCGT**C**ACGAATTAAGCAATTCGTAACGCATT-3'	C27, SEQ ID NO:61
	3'-GCAATGTTCAGGCA**A**TGCTTAATTCGTTAAGCATTGCGTAA-5'	A15, SEQ ID NO:62
	AATGCGTTACGAATTGCTTAATTCGTAACGGACTTGTAACG*	
6	5'-CGTTACAAG**C**CCGTCACGAATTAAGCAATTCGTAACGCATT-3'	C32, SEQ ID NO:63
	3'-GCAATGTTC**A**GGCAGTGCTTAATTCGTTAAGCATTGCGTAA-5'	A10 SEQ ID NO:64
	AATGCGTTACGAATTGCTTAATTCGTGACGACTTGTAACG*	
7	5'-CGTT**C**CAAGTCCGTCACGAATTAAGCAATTCGTAACGCATT-3'	C37 SEQ ID NO:65
	3'-GCAA**A**GTTCAGGCAGTGCTTAATTCGTTAAGCATTGCGTAA-5'	A5 SEQ ID NO:66
	AATGCGTTACGAATTGCTTAATTCGTGACGGACTTGAAACG*	

Duplex AB

5'-TGCACGGACCTCGAGAGAACCGAACGATCCGGATGTGATCATGGGACAGAATTCGCACACTGCACTCGACG ATAGTCT-3' (STRAND A, SEQ ID NO:67)

3'-TCTTGGCTTGCTAGGCCTACACTACTACCCTGTCTTAAGCGTGTGACGT-5' (STRAND B, SEQ ID NO: 68)

TGCAGTGTGCGAATTCTGTCCCATCATCACATCCGGATCGTTCGGTTCT*

Duplex AC

5'-TGCACGGACCTCGAGAGAACCGAACGATCCGGATGTGATCATGGGACAGAATTCGCACACTGCACTGCAC GATAGTCT-3' (STRAND A, SEQ ID NO:67)

3'-TCTTGGCTTGCTAGGCCTACACTACTACCCTGTCTTAAGCGTGTGACGTGACGTGCTATCAG-5' (STRAND C, SEQ ID NO:69)

GACTATCGTGCAGTGCAGTGTGCGAATTCTGTCCCATCATCACATCCGGATCGTTCGGTTCT*

Duplex AD

5'-TGCACGGACCTCGAGAGAACCGAACGATCCGGATGTGATCATGGGACAGAATTCGCACACTGCACTGCAC GATAGTCT (STRAND A, SEQ ID NO:67)

3'-ACGTGCCTGGAGCTCTCTTGGCTTGCTAGGCCTACACTACTACCCTGTCTTAAGCGTGTGACGT-5' (STRAND D, SEQ ID NO:70)

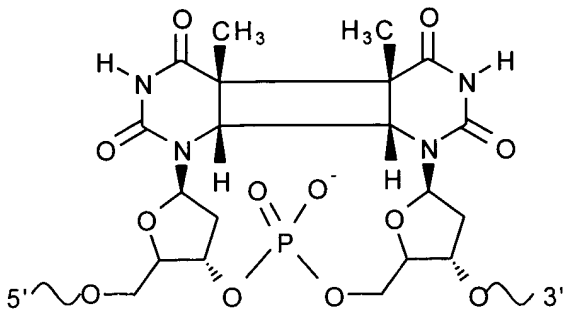
TGCAGTGTGCGAATTCTGTCCCATCATCACATCCGGATCGTTCGGTTCTCTCGAGGTCCGTGCA*

Duplex AE

5'-TGCACGGACCTCGAGAGAACCGAACGATCCGGATGTGATCATGGGACAGAATTCGCACACTGCACTGCACG ATAGTCT-3'(STRAND A, SEQ ID NO:67)

3'-ACGTGCCTGGAGCTCTCTTGGCTTGCTAGGCCTACACTACTACCCTGTCTTAAGCGTGTGACGTGAC GTGCTATCAGAG-5' (strand E, SEQ ID NO:71)

GAGACTATCGTGCAGTGCAGTGTGCGAATTCTGTCCCATCATCACATCCGGATCGTTCGGTTCTCTCGAGGT CCGTGCA*



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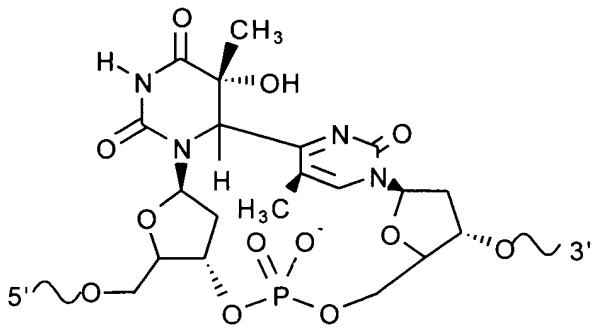
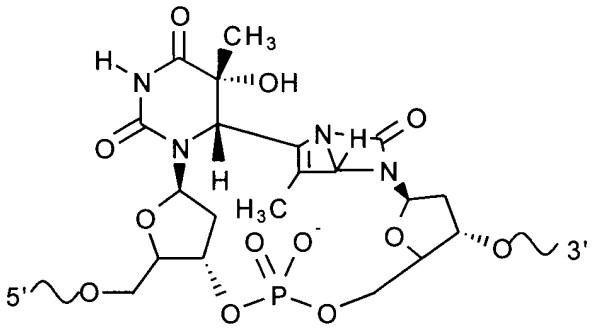


6-4PP



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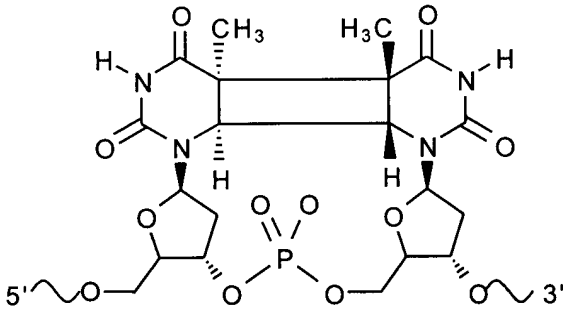

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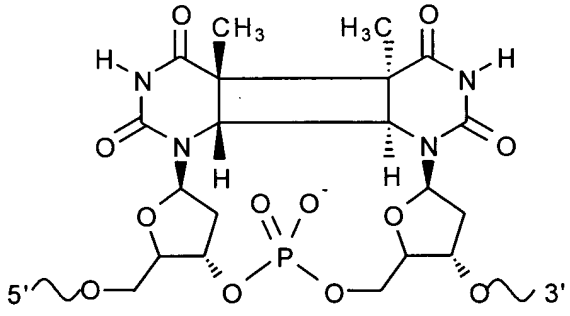

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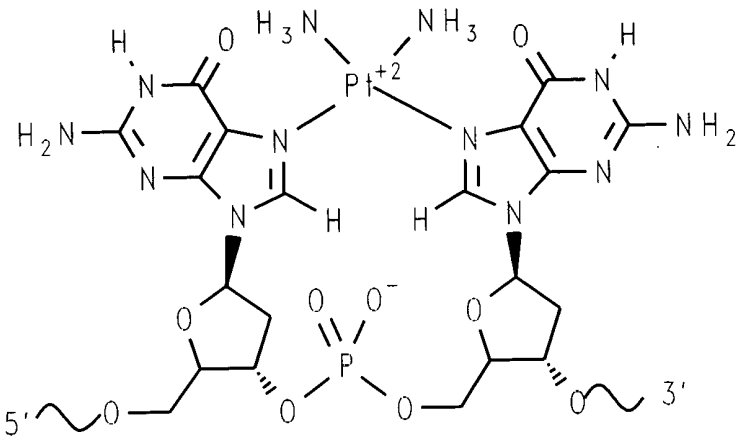
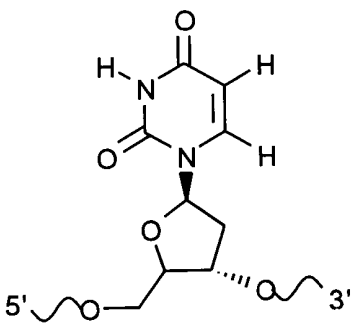
SCHEME 1A



SCHEME 1B

Pt-GG



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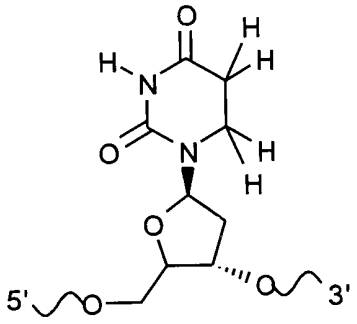

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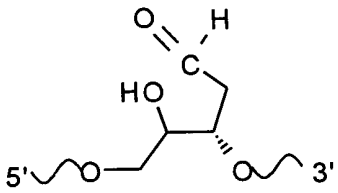

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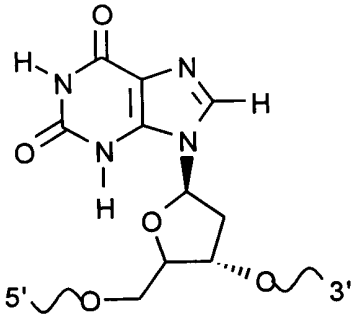

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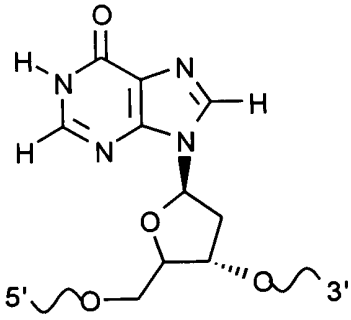

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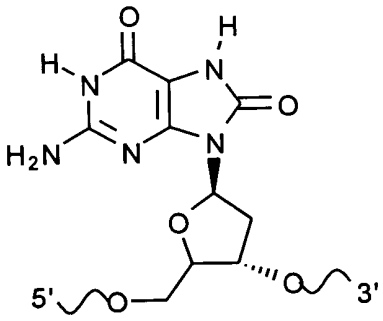

8-oxoG

SCHEME 1C